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Applicant SHANAHAN-PRENDERGAST, Elizabeth	

1. The designated Office is hereby notified of its election made:

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Patent

TITLE: THERAPEUTIC FORMULATIONS CONTAINING VENOM OR VENOM ANTI-SERUM EITHER ALONE OR IN COMBINATION FOR THE THERAPEUTIC PROPHYLAXIS AND THERAPY OF NEOPLASMS

INVENTOR: ELIZABETH SHANAHAN-PRENDERGAST.

The present invention comprises the method of treating a host organisms (man or animal) in need of a drug having direct or prophylactic anti-neoplastic activity comprising the administration of a therapeutically effective amount of Phospholipase A_2 targeted venom anti-serum alone or in combination with a known Phospholipase C anti-serum or a Phospholipase C inhibitory compound. A vaccine containing in whole or in part snake or insect venom or mammalian PLA_2 components comprising epitopes demonstrating Phospholipase A_2 activity and/or Phospholipase C enzyme components. This patent presents therapeutic pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which contain, total or partial, phospholipase A_2 enzyme activity or PLA_2 epitopes. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms and/or mammalian PLA_2 enzymes wherein the anti-serum has been preferably affinity purified for use in treating patients suffering from neoplastic disease. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect venoms or the PLA_2 enzyme components thereof and/or PLA_2 enzymes isolated from insect, mammalian on plant cells, and/or Phospholipase C enzyme preparation or extract from such venoms which may contain, total or partial, phospholipase A_2 enzyme activity.

In this patent the affinity purified anti-serum to venoms Phospholipase A_2 (PLA_2) and mammalian or plant PLA_2 are shown to be active anti -proliferative neoplastic agents.

The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom and/or other components of animal, insect or plant origin showing Phospholipase A_2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial, Phospholipase A_2 enzyme activity alone or in combination with animal or plant Phospholipase A_2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono or

polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the anti-serum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA_2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial, Phospholipase A_2 and C enzyme activity.

Phospholipase A_2 are lipolytic enzymes that hydrolyze the sn-2-acylester bond in glycerophospholipids. Many forms of PLA_2 exist in nature and have been described and classified into several groups. Types I, II and III PLA_2 are low molecular weight peptides (13-18 kDa) extra-cellular enzymes, including pancreatic and cobra venom PLA_2 (type I), rattle snake and inflammatory PLA_2 (type II) and bee venom type III. Intracellular cytosolic PLA_2 belong to different groups, including the 85 kDa (type IV) and 40-75 kDa enzymes.

Affinity purified anti-serum to venoms, animal or plant tissue demonstrating the ability to bind PLA_2 enzymes are shown herein below, by way of example, to be active in-vitro and in-vivo anti-proliferative neoplastic agents. Accordingly, these affinity purified antisera either alone or in combination with non-toxic Phospholipase C inhibitor or anti-serum to Phospholipase C are useful in the control of proliferation of neoplastic tissue.

BACKGROUND OF THE INVENTION

There is evidence to indicate that Phospholipase A_2 (PLA_2) is involved in the pathogenesis of many diseases. Thus local and circulating levels of Phospholipase A_2 enzyme and enzymatic products are elevated during infection, inflammatory diseases, tissue injury and brain dysfunction and is a very early indication of neoplastic development prior to tumour cell mass being evident by conventional methods of scanning tissue tumours.

Excessive Phospholipase A_2 activity may promote chronic inflammation, allergic reaction, tissue damage and pathophysiological complications. These effects may be the result of accumulating Phospholipase A_2 products (lysophospholipids and

free fatty acids, e.g. Arachidonic Acid) and destruction of key structural phospholipid membrane components, but are potentated by secondary metabolites, such as eicosanoids and platelet-activating factor. Phospholipase A_2 products or lipid mediators derived therefrom have been implicated in numerous activities that are an integral part of cell activation; chemotaxis, adhesion, degranulation, phagocytosis and aggregation.

Phospholipase A_2 secreted excessively at local sites may be responsible for tissue damage common to rheumatic disorders, alveolar epithelial injury of lung disease and reperfusion.

During acute myocardial ischemia, cytosolic Phospholipase A_2 and Phospholipase C activation causes increased intracellular Ca^{2+}. Subsequent accumulation of lysophospholipids and free fatty acids promote damage to sarcolemmal membranes leading to irreversible cell injury and eventually cell death.

Altered cytosolic Phospholipase A_2 and Phospholipase C activity or defects in their control and regulation is a predisposing factor to causing tumour cell development.

Prostaglandins and related eicosanoids are important mediators and regulators of both immune and inflammatory responses. Prostaglandin E_2 induces bone resorption and Leukotriene B_4 stimulates vascodilation and chemotaxis. Increased levels of Phospholipase A_2 is noted in Rheumatoid Arthritis (R.A.), osteoarthritis, gout, collagen and vascular diseases. Phospholipase A_2 induces non specific airway hyperactivity that is evident in asthma. Phospholipase A_2 is also elevated in peritonitis, septic shock, renal failure, pancreatis, Chrons and Graves Disease.

The activity of cell-mediated defence systems is stimulated by consecutive formation of interleukin 1β (IL-1β), interleukin-2 (IL-2) and interferon γ (IFN γ). The system is inhibited by interleukin-4 (IL-4) and also by prostaglandin E_2 (PGE_2) and histamine, which are released when the immune system is activated. The inhibition is strong in cancer patients, because PGE_2 is formed in many cancer cells and its formation is stimulated by IL-1β. PGE_2 and histamine are feedback inhibitors of cell mediated immunity.

PGE_2 is formed from arachidonic acid in monocytes, macrophages, cancer cells and other cells, when arachidonic acid is released from cellular phospholipids. The formation of PGE_2 is stimulated by several compounds, including histamine, IL-1 (α and β) and Tumour Necrosis Factor α (TNFα). PGE_2 inhibits the formation and receptor expression of IL-2 by increasing the level of cyclic AMP (cAMP) in helper T cells. This concomitantly decreases the formation of IFN γ.

PGE_2 inhibits the ability of natural killer cells (NK) to bind with tumour cells by increasing cAMP in Natural Killer Cells. This decreases tumour cell killing.

When the immune system is stimulated to destroy tumour cells, the killing is prevented because IL-1β stimulates PGE_2 formation in tumour cells, which increases cAMP levels in NK cells and prevents the binding of NK and tumour cells.

The activation of the cell-mediated defence is blocked also because PGE_2 increases cAMP in helper T cells and inhibits the formation of IL-2 and IFN γ.

Cytotoxic T cells can also produce PGE_2 thus inhibiting the activity of NK cells.

A number of human and experimental animal tumours contain and/or produce large quantities of prostaglandins (PG). Prostaglandins E_2 has been shown to effect significantly cell proliferation in tumour growth and to suppress immune responsiveness.

Phosphatidylinositol specific phospholipase C is an important enzyme for intracellular signalling. There are at least three major classes of Phosphatidylinositol specific Phospholipase C (PtdInsPLC: PtdInsPLC β, γ, δ). PtdInsPLCs hydrolyse a minor membrane phospholipid, phosphatidylinositol (4, 5) bisphosphate (PtdIns (4,5) P_2) to give the second messengers inositol (1, 4, 5) trisphosphate (Ins (1, 4, 5) P_3), which releases Ca++ from intracellular stores to increase the intracellular free CA++ concentration, and diacylglycerol which activates the Ca++ and phospholipid-dependent protein serine/threonine kinase, protein kinase C. Proteins phosphorylated by protein kinase C include transcription factors. Together, the increase in intracellular free Ca++ concentration and the activation of protein kinase C lead to a series of events that culminate in DNA synthesis and cell proliferation in tumour cells.

A number of growth factors and mitogens, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and bombesin, act through specific receptors to increase Ptd Ins PLC activity in cells. Continued stimulation of Ptd Ins PLC can lead to cell transformation.

Ptd Ins PLC activity is found to be increased in a number of human tumours. 76% of human breast cancers have detectable Ptd Ins PLC-γ immunoreactive protein compared to only 6% in benign breast tissue.

Cytosolic Ptd Ins PLC activity is increased up to >4-fold in human non-small cell lung cancer and renal cell cancer compared to normal tissue.

SUMMARY OF THE INVETION

The present invention comprises the method of treating mammals including humans in need of a drug to prevent neoplastic tissue growth and spread by the administration of a therapeutically effective amount of venom anti-serum prepared to whole venom or to parts of the venom or components of plant or animal origin which demonstrate PLA_2 activity. Also enhanced anti-cancer effects both in-vitro and in-vivo have been realised by combining this affinity purified anti-serum to PLA_2 components and/or mammalian PLA_2 with a non-toxic inhibitor of Phospholipase C or with anti-serum (polyclonal or monoclonal) developed to Phospholipase C enzyme.

This patent relates to the administration of one or more compounds which can generally be described as performing their function by either directly or indirectly causing Phospholipase A_2 and/or Phospholipase C enzyme inhibition, wherein the said inhibition is either partial or total. In addition this patent relates to the administration of one or more compounds which can generally be described as performing their function by interaction with the neoplastic cell membrane preventing their growth or spread, thus preventing further disruption of non-involved organs of the body and causing no toxicity to the infected patient or animal being treated.

Additional aspects of the invention relates to pharmaceutical compositions containing the compounds of the invention as active ingredients, modifying unwanted immune responses, and to methods of retarding proliferation of tumour cells using the compounds and compositions of this invention.

The anti-serum to snake venom PLA_2 and to plant, insect, mammalian and/or to PLA_2 epitopes or mimic molecules are shown herein to be active anti-tumour proliferative compounds and immune enhancing. For use in this regard, the compounds of the invention are administered to mammals, including humans, in an effective amount of 0.05 to 5 gms per day per kilogram of body weight. The amount depends, of course, on the condition to be treated, the severity of the condition, the route of administration of the drug, and the nature of the subject. The drugs may be administered IV, orally, parenterally, or by other standard administration routes including targeting with liposomes/RBC.

The therapeutic activity of the compounds of this invention are demonstrated by inhibition of the tumour cell lines in-vitro and in-vivo. The compounds were tested for toxicity in Scid mice. Results as in Figure A1.

Toxicity Study

Method

Female Scid mice (6-8 weeks of age) were treated with either a Neat or a 1:10 dilution of the anti-serum preparation, subcutaneously (0.1 ml, daily) for a period of 14 days. The weights of the mice were measured daily. At termination, organs were removed and fixed in formalin for histological examination.

Results

No toxicity, as assessed by animal weights and clinical well-being, was evident (Figure A1).

The compounds of this invention may be combined with other known anti-inflammatory/immunosuppressive or chemotherapeutic agents such as steroids or non-steroidal anti-inflammatory agents in the pharmaceutical compositions and methods described herein.

Anti-serum to snake and/or insect venoms and/or mammalian and/or PLA_2 enzyme or its epitopes can be used as a therapeutic treatment in diseases where elevated levels of Phospholipase A_2 are evident, (e.g. Rheumatoid Arthritis, see Fig. B). It is also envisaged that this novel therapy with anti-serum to venom PLA_2 (snake or insect) and/or to PLA_2 components (derived from animal or plant) can be applied as a prophylactic therapy by using sub-lethal doses of venoms or the venom PLA_2 enzyme extracts together with mammalian or plant PLA_2 or synthetic peptides demonstrating PLA_2 activity plus adjuvant to stimulate an immunoglobulin response within the patient, see results – Vaccine Efficacy in Balb/c mice. It is also envisaged that a synthetic peptide incorporating the Phospholipase A_2 and/or Phospholipase C activity could be used to generate said anti-serum or therapeutic agent or vaccine. Use may also be made in the generating of this therapeutic vaccine/anti-serum by using the known sequence homology that exists between human Phospholipase A_2 and snake/insect venoms together with animal PLA_2 used in combination with compounds known to inhibit Phospholipase C activity or anti-serum developed to this enzyme.

Sustained or directed release compositions can be formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable coatings, e.g. by microencapsulation, multiple coatings, etc.. It is also possible to freeze-dry the new compounds and use the lyophilizates obtained, for example, for the preparation of products for storage and subsequent injection.

EXPERIMENTATION

The compounds of this invention can be identified as anti-serum to snake or insect venoms mammalian or plant PLA_2 or parts thereof or Phospholipase C or mimic molecules generated to venoms or mammalian PLA_2 molecules and/or Phospholipase C or parts thereof also the pharmaceutical use of venoms or parts thereof and/or mammalian PLA_2 or enzyme components as vaccine antigen are

incorporated. Non-toxic compounds showing anti-phospholipase C activity can be incorporated with the anti-serum to PLA_2 of any origin, or mimic molecules demonstrating Phospholipase A_2 activity.

In certain applications of this therapy it may be necessary to curtail the ADCC reaction which could cause serum sickness and to ensure that this does not occur the IgG (FC) component is enzymatically cleaved from the affinity purified immunoglobulin so that natural killer cells will not react to the immunoglobulin in the anti-serum.

Ability of anti-serum to snake venom to inhibit Phospholipase A_2 enzyme isolated from human synovial fluid (Figure A2).

The inhibition of Phospholipase A2 enzyme from synovial fluid isolated from a patient with Rheumatoid Arthritis was tested with a range of dilutions of anti-serum to snake venom. Anti-serum to snake venom generated in horse, reconstituted in 10 ml sterile water. The following dilutions were used 1:10, 1:20, 1:40 and 1:60. The method used was as outlined in "Infection and Immunity, Sept. 1992, p. 3928-3931. Induction of Circulating Group II Phospholipase A_2 Expression in Adults with Malaria.

Results (Figure A2)

Dilution	Inhibition
1:10	63%
1:20	50%
1:40	35%
1:60	29%

In-vitro testing of un-affinity purified snake venom.

A range of tumour cell lines were tested with 3 concentrations of the anti-serum to snake venom by the MTT Assay. This anti-serum was not affinity purified. MTT Assay described by Alley et al, (Cancer Research, 48: 589-601, 1988) See Figure B.

SUMMARY OF RESULTS (Figure B)

Molt 4: Human T cell Lymphoma Cancer

Serum-containing

Dilution	**% of Control**
Neat	48.1
1:10	53.7
1:20	40.8
Serum-Free	
Neat	58.7
1:10	51.2
1:20	40.6

MDA 468: Human Breast Cancer

Serum-containing

Dilution	**% of Control**
Neat	8.0
1:10	53.7
1:20	58.9
Serum-Free	
Neat	15.4
1:10	48.4
1:20	58.9

C170HM2: Human Colon Cancer

Serum-containing

Dilution	**% of Control**
Neat	9.3
1:10	61.4
1:20	55.6
Serum-Free	
Neat	15.2
1:10	47.3
1:20	49.5

Pan 1: Human Pancreatic Cancer

Serum-Containing

Dilution	**% of Control**
Neat	9.3
1:10	47.5
1:20	49.2

Serum-Free

Neat	43.1
1:10	53.2
1:20	69.4

841: Human small cell lung cancer

Serum-containing

Dilution	**% of Control**
Neat	25.2
1:10	45.5
1:20	51.1

Serum-Free

Neat	63.4
1:10	60.1
1:20	59.8

T24: Human Bladder Cancer

Serum-containing

Dilution	**% of Control**
Neat	68.5
1:10	75.1
1:20	76.2

Serum-Free

Neat	84.1
1:10	87.9
1:20	88.4

Testing un-affinity purified anti-serum to Snake Venom against B16 F1 Melanoma Cell Line.

Mice

C57BL/6

Procedure

The mice were inoculated with 0.5 x 10^6 B16 F1 melanoma cells subcutaneously (sc) into flank region. Once palpable tumours had developed the mice received daily sc injections as follows:-

			number of mice
A	-	sterile water 100 µl	6
B	-	anti-serum (full strength) 100 µl	6
C	-	anti-serum (diluted 1:10) 100 µl	6

The dimensions of the tumours were taken daily using callipers. Once the tumours of the control mice were approximately 1.5 cm or larger in diameter all mice were killed. The tumours were removed and weighed.

Results

Small tumours were first discernible by palpitation in all mice 6-7 days after inoculation.

The changes in volume as measured by callipers, together with tumour weights at autopsy. See Fig. C for effect of anti-serum to snake venom on tumour growth retardation.

Effect of un-affinity purified anti-serum to snake venom on Melanoma B16F1 Growth

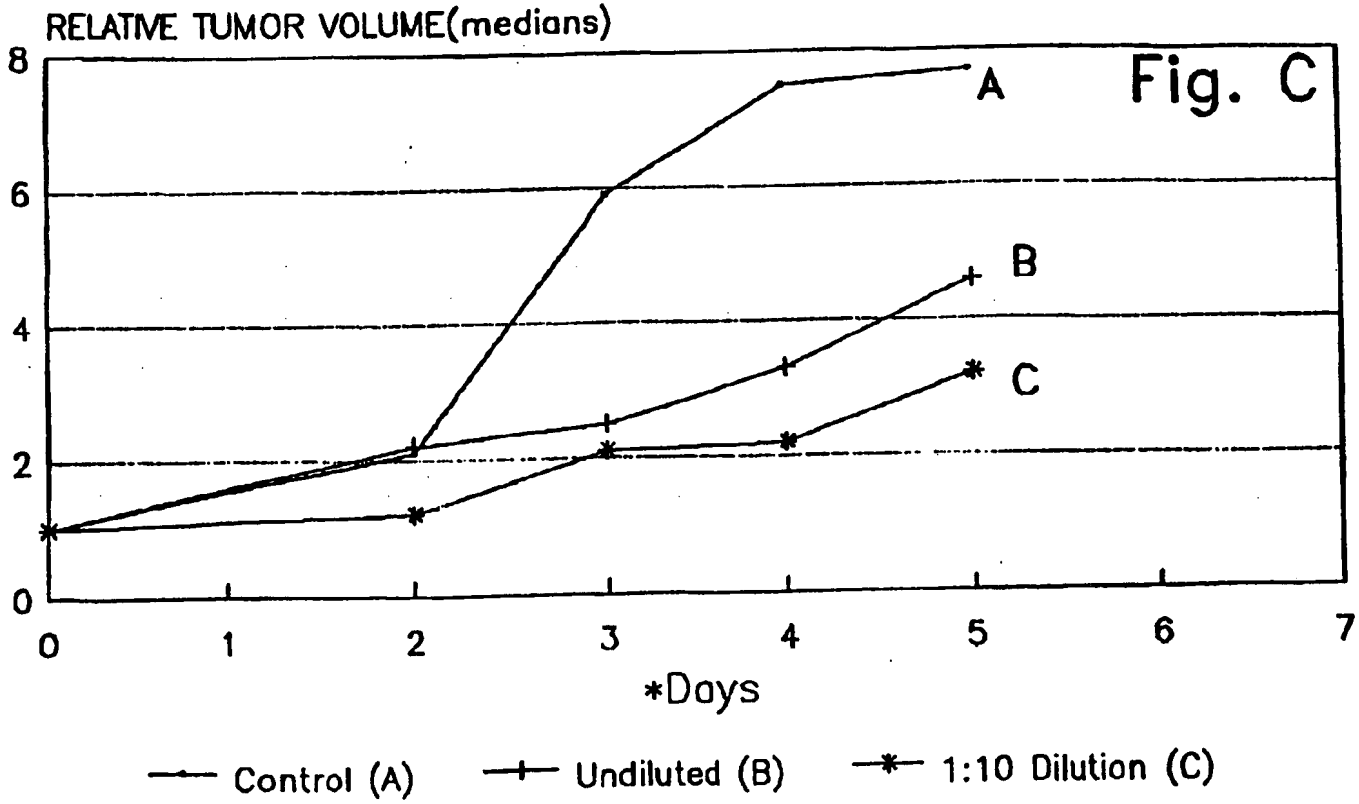


Fig. C

*Days are measured with day zero taken as day 7 post tumour inoculation.

IN-VITRO SREENING OF THE AFFINITY PURIFIED ANTI-SERUM TO SNAKE VENOM PREPARATION AGAINST A RANGE OF TUMOUR CELL LINES (Illustrated in Fig. D)

Introduction

The in-vitro inhibitory effects of the horse generated anti-serum to snake venom preparation, previously evaluated were obscured due to serum enhancement of tumour cell growth. Thus in the following assay, affinity purified anti-serum to snake venom was evaluated.

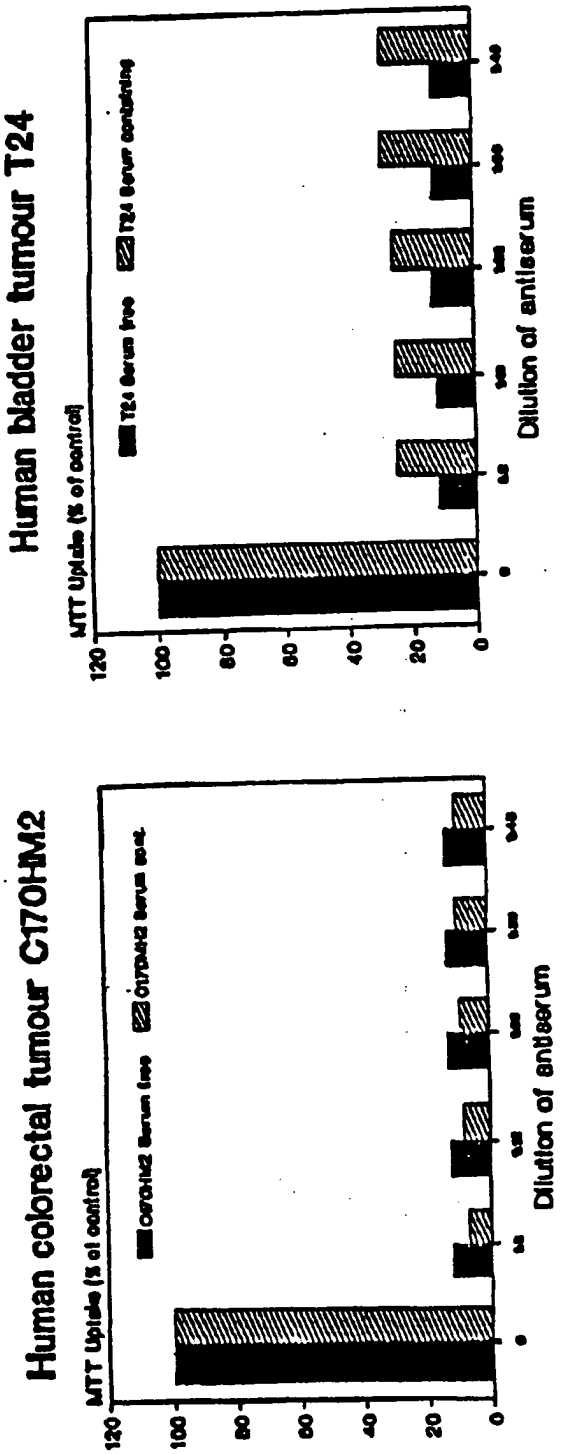
Method

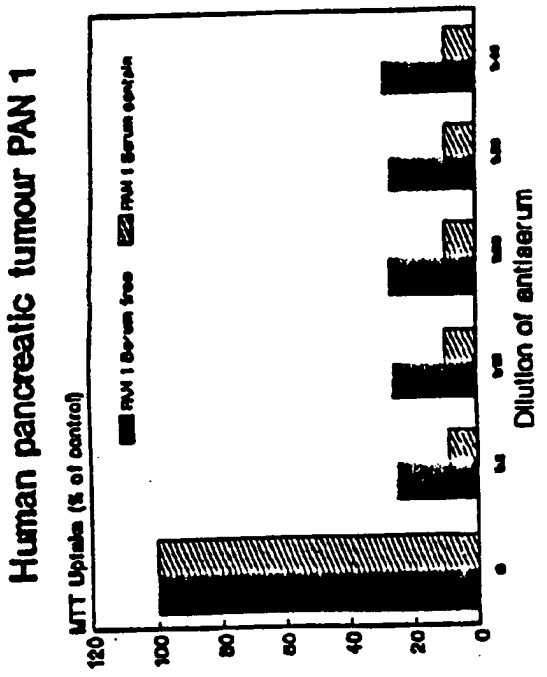
The cell lines were seeded into 96 well plates at a cell concentration of 10^4 cells per well in both serum free (Hams F12:RPMI 1640 + 0.5% bovine serum albumen) and serum-containing medium (RPMI 1640 + 10% heat inactivated foetal calf serum). The anti-serum preparation was diluted in the corresponding medium and added to the wells, 2-3 hours after the cells (to allow for cell adherence). The plates were incubated at 37°C in –5% CO_2 for 3 days. The cells were then incubated with 1 mg/ml MTT (methyl thiazol tetrazolium) for 4 hours at 37°C. The crystals were then solublised with dimethyl sulphoxide and the absorbance measured at 550nm.

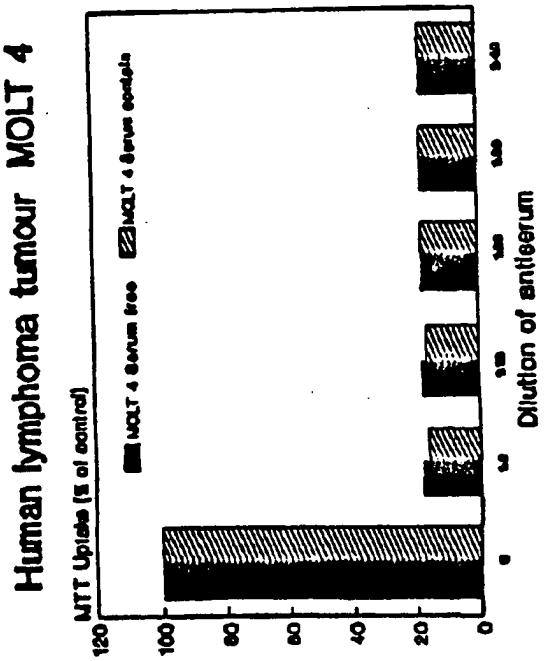
Results

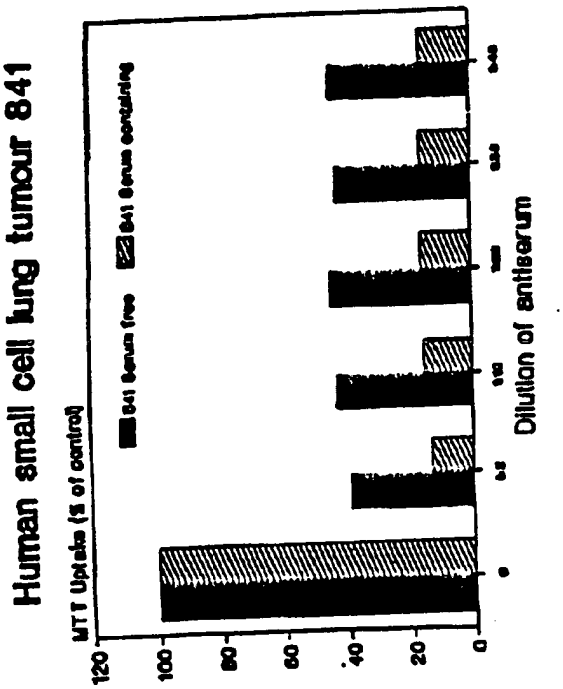
The test anti-sera inhibited all of the cell lines at all concentrations examined. The level of inhibition was statistically significant from the untreated control at all anti-serum dilutions, with all cell lines as assessed by a one way analysis of variance.

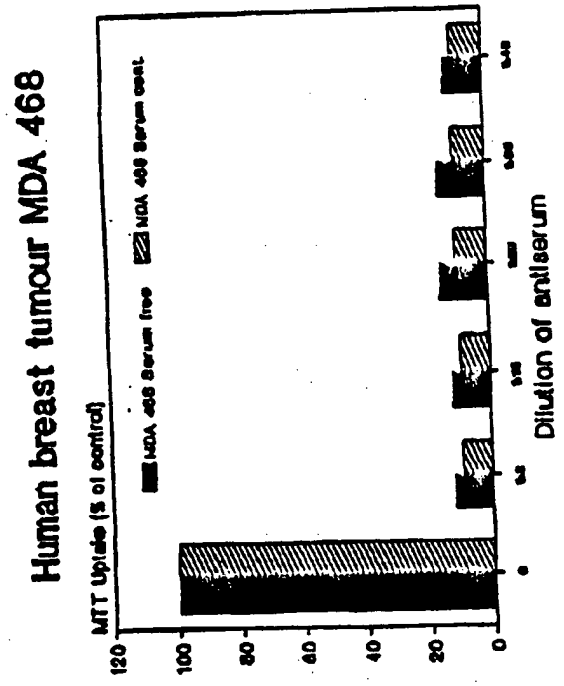
Figure D

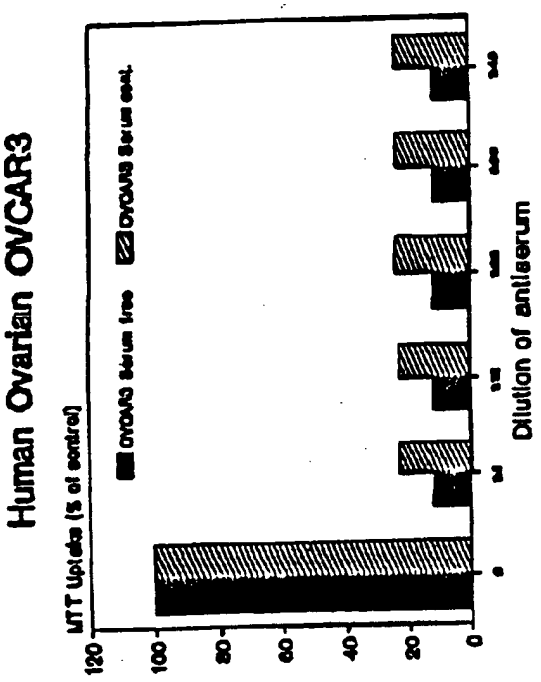

Human bladder tumour T24
MTT Uptake (% of control)
T24 Serum free
T24 Serum containing
120
100
80
60
40
20
0
Dilution of antiserum
Human colorectal tumour C170HM2
MTT Uptake (% of control)
C170HM2 Serum free
C170HM2 Serum cont.
120
100
80
60
40
20
0
Dilution of antiserum

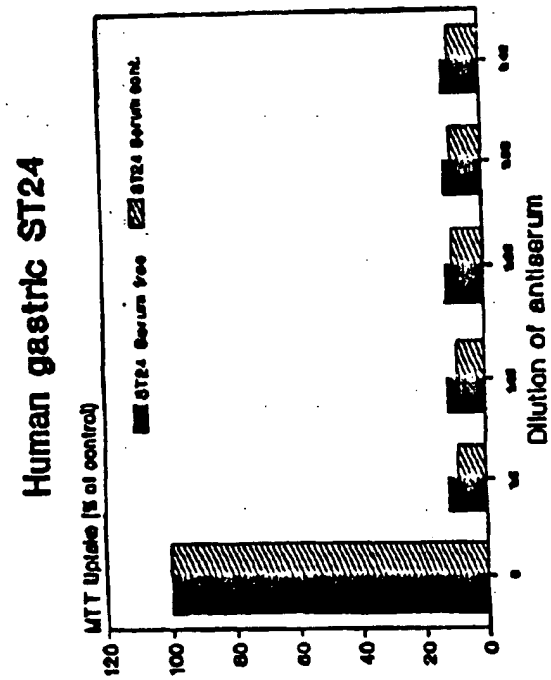

Human pancreatic tumour PAN 1
MTT Uptake (% of control)
PAN 1 Serum free
PAN 1 Serum contain
120
100
80
60
40
20
0
Dilution of antiserum


Human lymphoma tumour MOLT 4
MTT Uptake (% of control)
MOLT 4 Serum free
MOLT 4 Serum contain
120
100
80
60
40
20
0
Dilution of antiserum


Human small cell lung tumour 841
MTT Uptake (% of control)
841 Serum free
841 Serum containing
120
100
80
60
40
20
0
Dilution of antiserum


Human breast tumour MDA 468
MTT Uptake (% of control)
MDA 468 Serum free
MDA 468 Serum cont.
120
100
80
60
40
20
0
Dilution of antiserum


Human Ovarian OVCAR3
MTT Uptake (% of control)
OVCAR3 Serum free
OVCAR3 Serum cont.
120
100
80
60
40
20
0
Dilution of antiserum


Human gastric ST24
MTT Uptake (% of control)
ST24 Serum free
ST24 Serum cont.
120
100
80
60
40
20
0
Dilution of antiserum

IN-VIVO TEST

The effects of affinity purified anti-serum to snake venom on human colorectal C170HM$_2$ cell line.

Materials and Methods

C170MH$_2$ cells were injected subcutaneously into the left flank of ten male nude mice. The mice were allocated randomly to two groups.

Group 1 – 100 µl anti-serum twice daily intravenously (IV)

Group 2 – 100 µl PBS twice daily IV

Tumours were measured twice weekly, using callipers, in two dimensions. Cross-sectional areas were calculated. The mice were also weighed once weekly. The therapy was terminated at day 22.

Results

The cross-sectional areas were measured at increasing time points during the experiment, as shown in Fig. E. The affinity purified anti-serum preparation induced a slowing in growth when compared to saline controls. An ANOVA was performed on the results in which the treatment was evaluated with respect to time, and shows a significance of $P = 0.028$.

At the termination of the experiment, the tumours were weighed and the results are shown in Fig. F. No toxic effect of the affinity purified anti-serum preparation was observed.

Figure E: Effect of affinity purified anti-serum to snake venom on the mean cross-sectional area of C170HM2 in nude mice

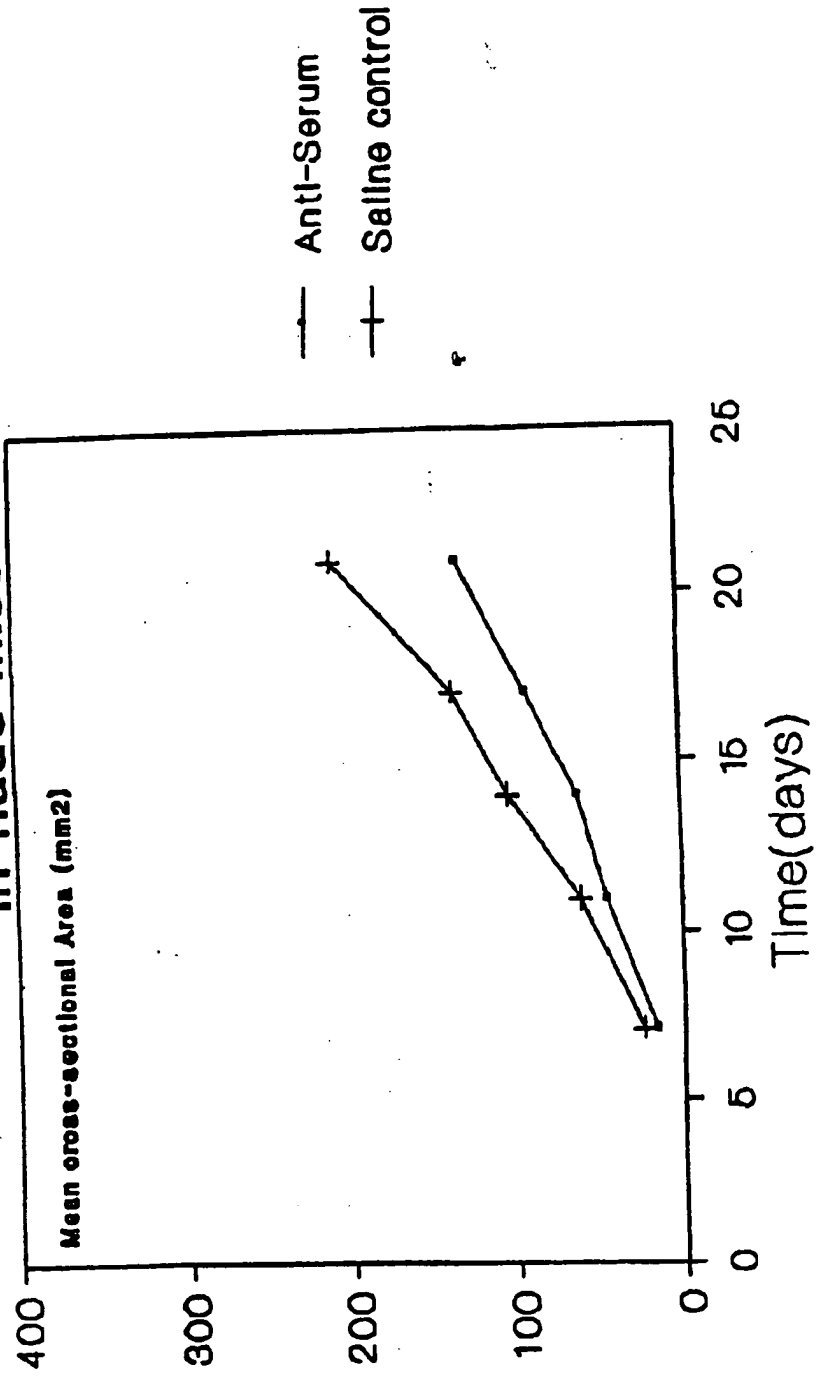
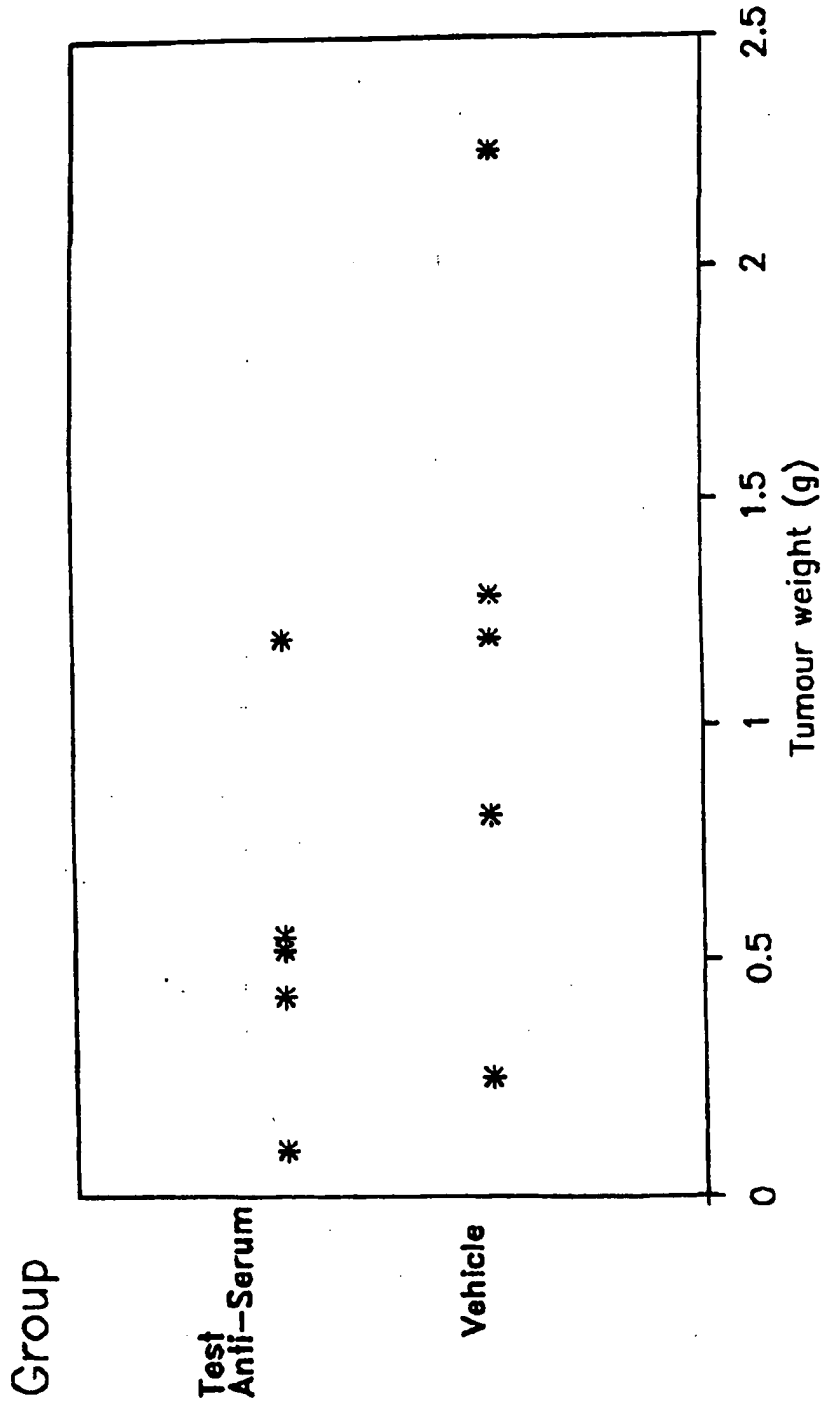


Figure F: Effect of affinity purified anti-serum to snake venom on the final tumour weight of C170HM2



In-vitro screen of the affinity purified anti-serum to snake venom preparation in combination with a phospholipase C inhibitor 1-oleoyl-2-acetyl-sn-glycerol (OAG) 5μ molar, on a range of cancer cell lines.

Methods

The affinity purified anti-serum to snake venom preparation was diluted 1:2 and 1:10 and was combined with 5μ molar OAG and added to the wells as previously described for the MTT Assay. The cell lines tested were Human Breast tumour, MDA 468, Human small cell lung tumour 841 and Human renal TK-10. Results as shown in Fig. G

Figure G: Affinity purified anti-serum to snake venom and (OAG) a Phospholipase C inhibitor combination.

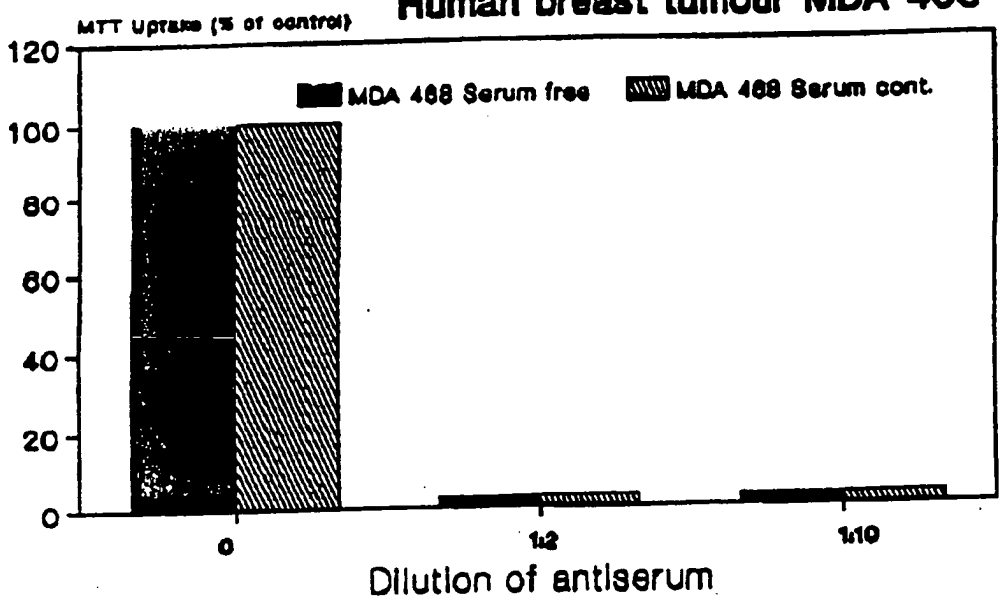


Figure G: Affinity purified anti-serum to snake venom and (OAG) a phospholipase C inhibitor combination

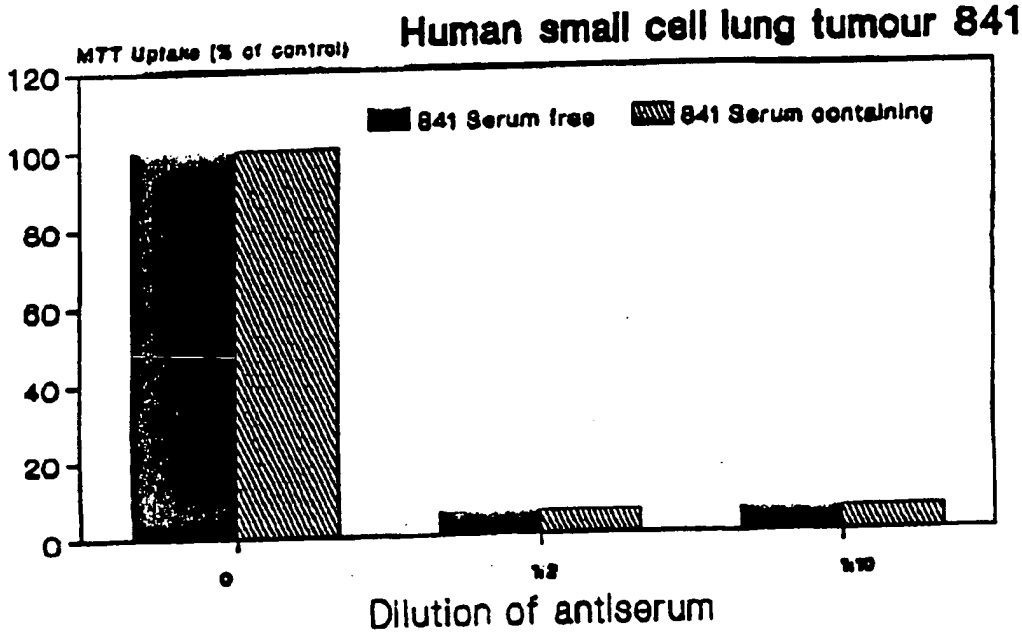
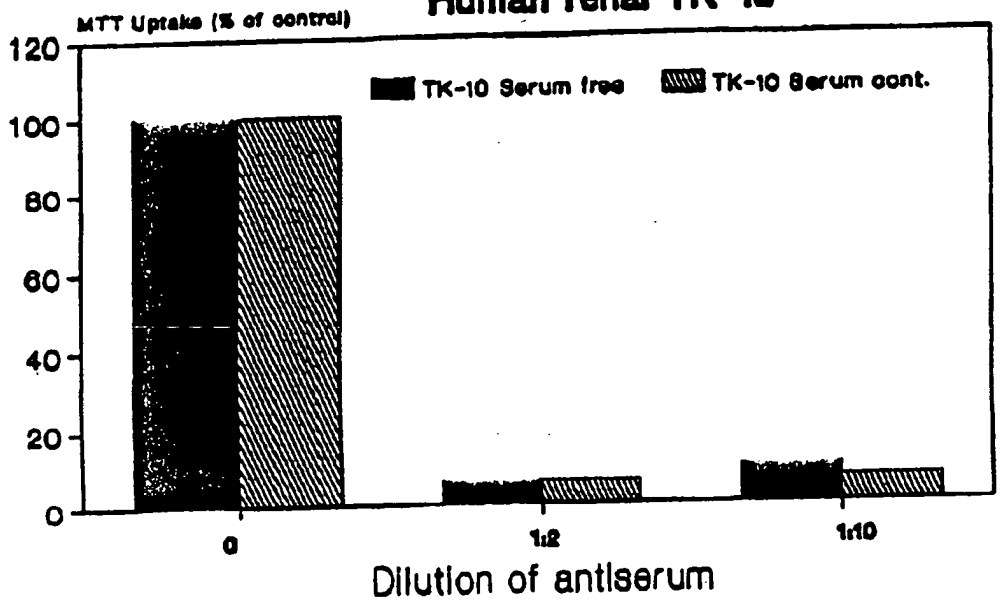


Figure G: Affinity purified anti-serum to snake venom and (OAG) a phospholipase C inhibitor combination

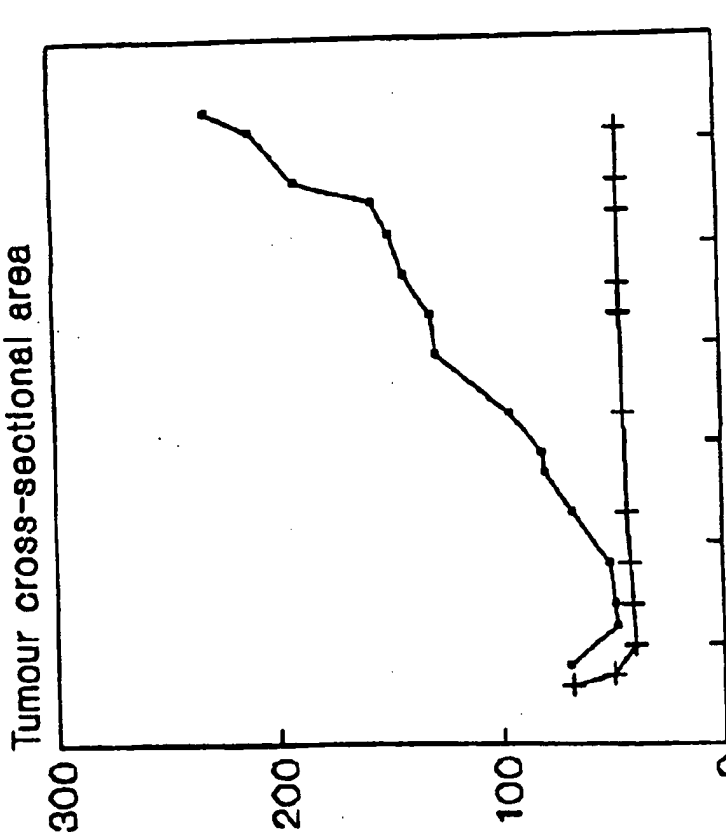


In-vivo testing of the combination of affinity purified anti-serum to snake venom and 1-oleoyl-2-acetyl-sn-glyceral (OAG) at 5μm concentration on the growth of MDA 468 cell line.

Method

MDA 468 tumours were aseptically removed from donor female Scid mice. The tissue was aseptically minced, pooled and implanted into anaesthetised female Scid mice (anaesthetic comprised of a 0.2 ml injection of Hypnorm (Jannsen) :Hyonovel (Roche) :distilled water in a 1:1:5 ratio). Tissue implants consisted of 3-5 mm^2 pieces and after subcutaneous transplantation into the left flank, the incision was clipped. The Scid mice were then randomised into 2 groups of 10 animals. They were treated daily with a 0.2 ml subcutaneous injection (in the opposite flank to the tumour graft) of a combination of affinity purified anti-serum to snake venom and 5μm molar of (OAG) dilution of the anti-serum preparation. The control animals received 0.2 ml phosphate buffered saline, pH 7.6. All animals were terminated on day 63, and the tumours were dissected out, weighed and processed for histology. Results are in Fig. H.

Figure H:Effect of the affinity purified anti-serum to venom in combination with the Phospholipase C inhibitor (OAG) 5µm



Vaccine Efficacy in Balb/c mice after challenge with WEHI-3 cell.

The objective of study is to demonstrate the efficacy of sub-lethal levels of Russelli vipera venom entrapped in liposomes and porcine phospholipase A_2 enzyme entrapped in liposomes working in combination to confer a sustained and protective antibody response to a challenge by Leukaemia cells (WEHI-3 cells)

The Russelli vipera venom was toxoided with 2% osmium tetroxide and entrapped in liposomes (egg phosphocholine and cholesterol). The liposomes were sterilised.

The Porcine Phospholipase A_2 enzyme was entrapped in liposomes (egg phosphocholine and cholesterol) and were sterilised.

Immunisation of mice consisted of an initial subcutaneous injection of 0.25 mls (containing 250μg of venom) and 3 days later the mice were injected subcutaneously with 0.25 mls of porcine PLA_2 (containing 250 μg of porcine PLA_2. Boosters of each vaccine were given at 3 week intervals.

Control mice were injected with 0.25 mls of sterile physiological saline on days corresponding to test mice inoculations.

Animals

Balb/c mice (20-25 g) were used in the study. 15 mice were used in each group.

Group I - test mice
Group II - control mice

Challenge

The immunised mice and controls were challenged by intravenous injection into tail vein with approximately 5×10^5 leukemic cells (WEHI-3 cells) on day 30 of study.

Test mice are observed for extended life span after the death of the control mice after approximately 24 days.

Results Obtained

All control mice died of leukaemia within the allotted time span of 24 days. The venoid combination inoculation protected the vaccinated group from the cancer cell challenge and there was a 100% survival rate at day 35 when the experiment was terminated.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilise the present invention to its fullest extent. The preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the disclosure in any way whatsoever.

I Claim

1. A method of treating neoplasm in a mammal in need of such treatment, comprising administering to said mammal a therapeutic agent comprising venom and/or mammalian, plant or insect anti-serum reactive with at least one Phospholipase A_2 enzyme.

2. A method according to claim 1 wherein the anti-serum is reactive with two or more Phospholipase A_2 type enzymes.

3. A method according to claim 1 wherein the at least one Phospholipase A_2 Type enzyme is Type I, Type II, Type III or Type IV.

4. A method according to claim 1 wherein the anti-serum is either polyclonal or monoclonal.

5. A method of treating a mammal prophylactically to prevent neoplastic development, comprising administering to said mammal a therapeutic vaccine containing venom and/or mammalian, plant or insect PLA_2 enzymes or part thereof as the principal antigen component.

6. A pharmaceutical formulation containing venom and/or mammalian plant or insect anti-serum to PLA_2 enzyme or part thereof in combination with anti-serum to Phospholipase C enzyme or part thereof or inhibitory compounds to Phospholipase C for use as a therapeutic agent for the therapy of a neoplastic condition in a human or animal.

7. A method according to claim 6 wherein the inhibitory compounds to Phospholipase C is one or more of EDTA, Phenanthroline, Chloromercuribenzoic Acid, Iodoacetic Acid, and 1-oleoyl-2-acetyl-sn-glycerol(OAG).

8. A pharmaceutical formulation containing one or more venoms or venom components as antigen and/or mammalian, plant or insect PLA_2 enzyme as antigen in combination with Phospholipase C enzyme.

9. A method according to Claim 8 wherein the phospholipase C enzyme inhibitor is used in combination with the therapeutic agents of Claim 1 to enhance anti neoplastic and anti metastatic activity.

10. A method according to any one of Claims 1, 5, 6 and 8, wherein the administration is part of a combination therapy with other therapeutically effective agents.

11. A method according to Claims 1, 5, 6 and 8 wherein the administration is in combination with adjuvants.

12. A method according to Claims 1, 5, 6 and 8 wherein the venom is that of snake and/or insect.

13. A method according to Claims 1, 5, 6 and 8 wherein the Phospholipase A_2 enzyme showing Phospholipase A_2 activity is obtained from more than one species of snake and/or insect, mammal or plant.

14. A method according to Claims 1, 5, 6 and 8 wherein the therapeutic agent is administered as an anti-inflammatory agent.

15. A method according to Claims 1, 5, 6 and 8 wherein the therapeutic agent is administered to prevent the occurrence of immunosuppression.

16. A method according to Claims 1, 5, 6 and 8 wherein the therapeutic agent is administered in the treating of allergic contact dermatitis, Asthma and Psoriasis and bronchitis.

17. A method according to Claims 1, 5, 6 and 8 wherein the anti-serum is administered for the treatment of physiological condition resultant from elevated levels of phospholipase A_2 products and/or metabolites.

18. A method according to claim 17 wherein the physiological condition is Schizophrenia.

19. A method according to Claims 1, 5, 6, 8 and 17 wherein the anti-serum to Phospholipase A_2 and/or C are produced synthetically by molecular imprinting of template organic molecules using these enzymes.

20. Therapeutic agents according to Claims 1, 5, 6 and 8 for treating one or more of the following:- Rheumatoid arthritis, osteoarthritis, gout, rheumatic carditis and autoimmune diseases, allergic diseases, bronchial asthma, septic shock, renal failure, pancreatis, myasthenia gravis and ocular and dermal inflammatory diseases, psoriasis, splenomegaly, cancer, metastatic spread of neoplasm, collagen vascular disease, myocardial ischemia, cellular chemotaxis, depression, erythema, vascular permeability resultant from enhanced production of PGE_2, acne, atopic diseases, malaria, allergic conjunctivitis, schizophrenia, reiters syndrome, raynaud's phenomenon, lupus, Chron's and Graves disease.

21. A method according to Claims 1, 5, 6, 8 and 17 wherein the Fc receptor of the antibody to either Phospholipase A_2 and C used in this therapeutic method is either totally or partially removed.

22. A method according to Claims 6, 8, 19 and 21 wherein a non-toxic compound demonstrating inhibiting activity against Phospholipase C enzymes may be utilised in conjunction with the PLA_2 anti-serum to enhance its anti-neoplastic (tumour) and anti-metastatic activity.

23. A method according to Claims 1, 5, 6, 8,17 and 19 wherein the anti-serum is generated to human Phospholipase A_2 enzyme either in a mono and/or polyclonal form.

24. A method according to Claims 1, 5, 6, 8 and 17 wherein the anti-serum to Phospholipase A_2 enzyme is generated in eggs, producing antibodies which do not react with the human Compliment system.

25. A method according to Claims 1, 5, 6, 8 and 17 wherein the anti-serum to venom, mammalian, plant or insect Phospholipase A_2 is generated in mammals and extracted from the colostrum and preferably but not essentially affinity purified for use in oral administration to patients either alone or in combination with anti-serum similarly produced to human Phospholipase C enzyme components.

26. A method of inoculation of human or animal with a combination of two or more phospholipase A_2 enzymes types.

27. A method according to claim 26 where the antibody response to the inoculation confers prophylactic and/or therapeutic benefit to patient.

28. A method according to claim 27 wherein the patient is in need of a treatment for a neoplastic condition.

29. A method according to claims 26, 27 and 28 wherein the phospholipase A_2 type is Type I, Type II, Type III or Type IV.

30. A method according to claim 29 wherein the Phospholipase A_2 is obtained from venom.

31. A method according to claim 29 wherein the Phospholipase A_2 is obtained from animal or plant species.

32. A method according to claim 1, 5, 6, 8 and 26 wherein the phospholipase A_2 is synthetically produced or cloned.

33. A method of early detection of neoplastic disease by utilising the detection of enhanced PLA_2 levels in patients.

34. A method according to claim 33 wherein the detection of enhanced PLA_2 is established by the use of Lipose Diagnostic Kit.

35. A method according to claims 2, 26, 27 and 28 wherein Phospholipase A_2 type enzyme has a size of between 40-80 kDa.

36. A method of targeting cancer cells by use of Type I and/or Type II PLA_2 as targeting agent with hydrophilic tail.

37. A method according to claim 36 wherein the targeting agent is a liposome containing anti-serum to PLA_2 or conventional chemotherapy drugs.

38. A method treating parasitic and bacterial infections in mammals by the administration of a therapeutic agent containing venom and/or mammalian, plant or insect anti-serum reactive with Phospholipase A_2 enzymes

39. A method according to Claim 38 wherein the anti-serum is reactive with one or more Phospholipase A_2 type enzymes

40. A method according to Claim 39 wherein the Phospholipase A_2 Type enzymes is one of Type I, Type II, Type III or Type IV.

41. A method according to Claim 38 wherein said parasite is an haemoflagellate parasite.

42. A method as recited in Claim 41 wherein said parasite is a member of the group of haemoflagellate parasites consisting of Leishmania, Trypanosomia and Toxoplasma.

ABSTRACT

The present invention comprises the method of treating a host organisms (man or animal) in need of a drug having direct or prophylactic anti-neoplastic activity comprising the administration of a therapeutically effective amount of Phospholipase A_2 targeted venom anti-serum alone or in combination with a known Phospholipase C anti-serum or a Phospholipase C inhibitory compound. A vaccine containing in whole or in part snake or insect venom or mammalian PLA_2 components comprising epitopes demonstrating Phospholipase A_2 activity and/or Phospholipase C enzyme components. This patent presents therapeutic pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which contain, total or partial, phospholipase A_2 enzyme activity or PLA_2 epitopes. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms and/or mammalian PLA_2 enzymes wherein the anti-serum has been preferably affinity purified for use in treating patients suffering from neoplastic disease. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect venoms or the PLA_2 enzyme components thereof and/or PLA_2 enzymes isolated from insect, mammalian on plant cells, and/or Phospholipase C enzyme preparation or extract from such venoms which may contain, total or partial, phospholipase A_2 enzyme activity.

In this patent the affinity purified anti-serum to venoms Phospholipase A_2 (PLA_2) and mammalian or plant PLA_2 are shown to be active anti -proliferative neoplastic agents.

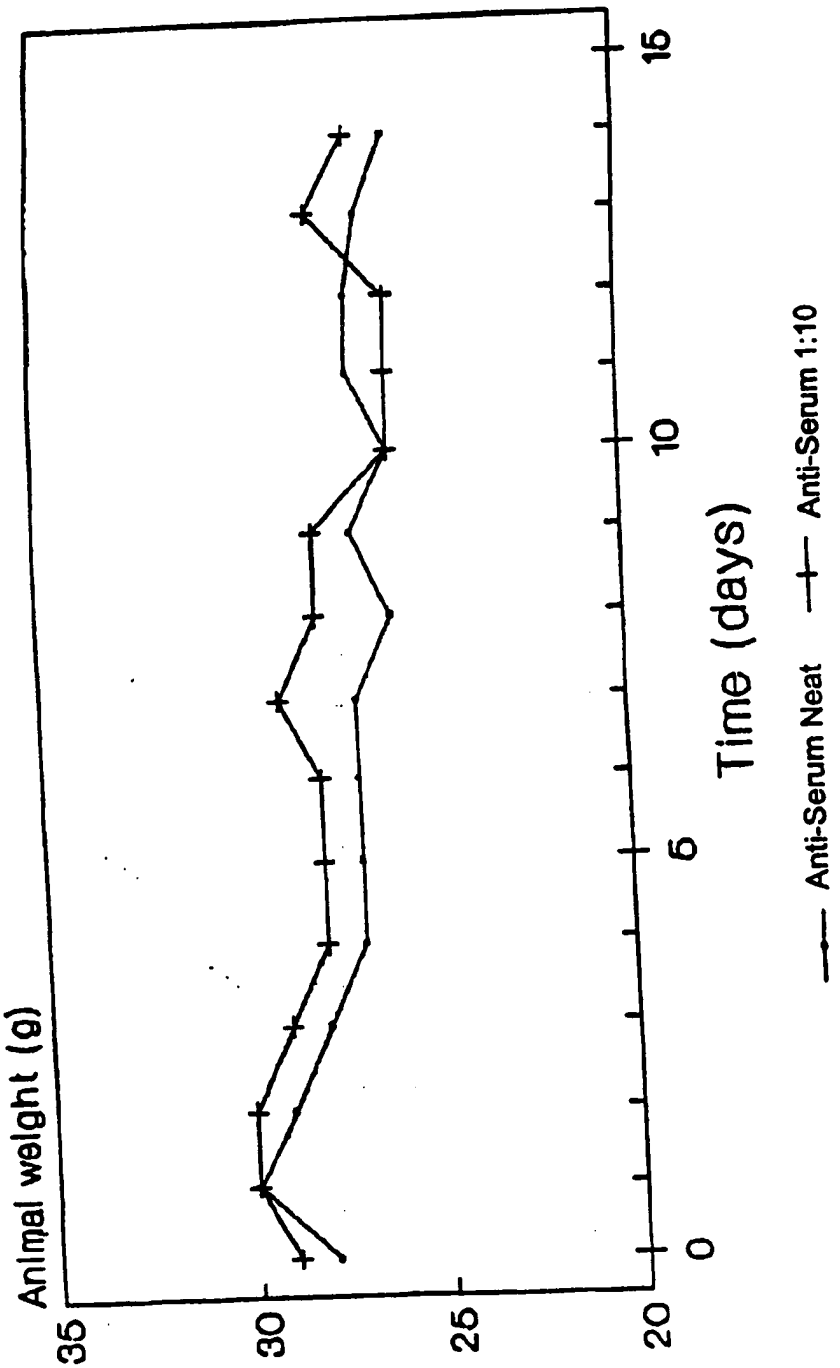
The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom and/or other components of animal, insect or plant origin showing Phospholipase A_2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial, Phospholipase A_2 enzyme activity alone or in combination with animal or plant Phospholipase A_2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono or

polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the anti-serum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA_2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial, Phospholipase A_2 and C enzyme activity.

Phospholipase A_2 are lipolytic enzymes that hydrolyze the sn-2-acylester bond in glycerophospholipids. Many forms of PLA_2 exist in nature and have been described and classified into several groups. Types I, II and III PLA_2 are low molecular weight peptides (13-18 kDa) extra-cellular enzymes, including pancreatic and cobra venom PLA_2 (type I), rattle snake and inflammatory PLA_2 (type II) and bee venom type III. Intracellular cytosolic PLA_2 belong to different groups, including the 85 kDa (type IV) and 40-75 kDa enzymes.

Affinity purified anti-serum to venoms, animal or plant tissue demonstrating the ability to bind PLA_2 enzymes are shown herein below, by way of example, to be active in-vitro and in-vivo anti-proliferative neoplastic agents. Accordingly, these affinity purified antisera either alone or in combination with non-toxic Phospholipase C inhibitor or anti-serum to Phospholipase C are useful in the control of proliferation of neoplastic tissue.

Figure A1
Toxicity Data



PATENT COOPERATION TREA

09/254623

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	15.12.98
Applicant's or agent's file reference F8009-7004	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB97/01091	10/09/1997	11/09/1996

Applicant
SHANAHAN-PRENDERGAST, Elizabeth

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
F8009-7004		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB97/01091	10/09/1997	11/09/1996
International Patent Classification (IPC) or national classification and IPC		
A61K35/58		
Applicant		
SHANAHAN-PRENDERGAST, Elizabeth		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 28 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/03/1998	15.12.98
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Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB97/01091

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-15	as received on	23/01/1998	with letter of	23/01/1998

Claims, No.:

1-42	as received on	23/01/1998	with letter of	23/01/1998

Drawings, sheets:

1/9-9/9	as received on	23/01/1998	with letter of	23/01/1998

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13,15,18-19,22-32,34,37-42
	No:	Claims	14,16,17,20,21,33,35,36
Inventive step (IS)	Yes:	Claims	5-9,18,22,26-28
	No:	Claims	1-4,10-17,19,20-21,23-25,29-42
Industrial applicability (IA)	Yes:	Claims	1-42
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

The documents which are referred to in this communication are numbered in the same order as cited in the International Search Report.

Section V:

V.1. *Novelty:*

V.1.1. The subject-matter of the claims 14, 16, 17, 20, 21 is not novel (Art. 33 (1) and (2) PCT) for the following reasons.

D 5 already discloses monoclonal antibodies (mAb) and fragments thereof to type I Phospholipase A_2 (PLA_2) for the treatment of inflammatory reactions, e.g. acute pancreatis (c.f. claims 14, 17, 20, 21).

D 6 shows the use of inhibitors of the function of murine and human PLA_2 such as antibodies to PLA_2 for the therapeutic treatment of inflammatory reactions, e.g. rheumatoid arthritis (c.f. claim 20 of the present application), psoriasis (claims 16, 20), asthma (claims 16, 20).

V.1.2. The subject-matter of the claims 33 and 35 is not novel (Art. 33 (1) and (2) PCT), since D 4 discloses the use of mAb recognizing membrane PLA_2 for the diagnosis of cancer via the measurement of PLA_2 level (see column 2, lines 1-32). Moreover, the subject-matter of claim 36 is not novel, since D 1 discloses PLA_2 and fragments thereof from snake venom used for detection of tumoural cells (see column 5, lines 29 ff.).

V.2. *Inventive Step:*

V.2.1. The subject-matter of the claims 1-4, 15, 23, 24, 39 does not involve an inventive step according to Art. 33 (1) and (3) PCT for the following reasons.

D 4 shows the use of mAb to human membrane PLA_2 for the diagnosis of cancer.

D 6 discloses the use of inhibitors of the function of murine and human PLA_2 such as antibodies to PLA_2 for the treatment of inflammatory reactions, but also of other diseases mediated e.g. by prostaglandins (see column 15, line 16).

Prostaglandins like PGE_2 are known inhibitors of cell mediated immunity which have an effect on cell proliferation in tumour growth by decreasing tumour cell killing according to the description of the present application (page 4, line 10- to page 5, first paragraph).

Therefore, the man skilled in the art would certainly derive from D 6 in combination with D 4, that antiserum to PLA_2 derived from mammalian or other origin (claim 3) is useful in the treatment of neoplasm in a mammal (claim 1).

The same applies to the subject-matter of claim 15.
Moreover, it is well-known in the art that anti-serum (especially polyclonal) often reacts with more than one types of one enzyme (see claims 2, 39).
Furthermore, the man skilled in the art would expect to be successful in using either polyclonal or monoclonal antiserum (claims 4, 23) or antiserum generated in eggs, since the technique of generating antibodies in eggs is widely accepted in the art of immunology (claim 24).

V.2.2. The subject-matter of the claims 10 and 11 is not inventive (Art. 33 (1) and (3) PCT), since the man skilled in the art would certainly try to use the claimed therapeutic agents combined with other therapeutically effective agents such as other known antiinflammatory/immunosuppressive or chemotherapeutic agents (see page 7 of the description) and expect to be successful especially as there is no surprising effect recognizable from the description. The combination with adjuvants is obvious since the combining of an active agent with adjuvants is commonly employed in the state of the art.
The use of venom originating from snake according to claim 12 is derivable for instance from D 1, D 2, D 3 and does not involve an inventive step.
Furthermore, as it is known that venom (for instance cobra venom) contains PLA_2 and that for instance mammalia including humans contain these lipolytic enzymes (see page 2 of the present application), the subject-matter of the claims 30 and 31 is obvious for the skilled person.
The use of more than one species of snake according to claim 13 is also not inventive, since D 1 already shows a basic protein, namely a PLA_2 isolated from snakes, namely from Naja nigricollis and / or from Naja mossambica pallida (see abstract).

V.2.3. The subject-matter of the claims 19 and 32 is not inventive (Art. 33 (1) and (3) PCT) , since the synthetic productions of anti-sera and of enzymes are well-known techniques in the art, see for instance D 6, which mentions that PLA_2 enzymes can be purified from cell sources or produced recombinantly or synthetically (see column 14, lines 35-37).

V.2.4. Even if the subject-matter of claim 20 is rendered novel, it would not involve an inventive step according to Art. 33 (1) and (3) PCT with respect to the treatment of all diseases which are linked to inflammatory processes such as rheumatoid arthritis,

osteoarthritis, gout, rheumatic carditis, allergic diseases, ocular and dermal inflammatory diseases, acne, allergic conjunctivitis, Graves disease, Lupus, Reiter-Krankheit, since the man skilled in the art regarding D 6 would easily find out the special kinds of inflammatory diseases for which the claimed therapeutic agents are best suited.
Also the subject-matter of the claims 38, 41, 42 does not involve an inventive step, since the skilled man in the art would consider D 6, which discloses the use of inhibitors of the function of murine and human PLA_2 such as antibodies to PLA_2 for the treatment of inflammatory reactions, and derive that these therapeutic agents of D 6 would also be useful in the treatment of inflammatory reactions resulting from parasitic and bacterial infections.

V.2.5. The subject-matter of claim 25 does not include an inventive step, since the extraction of antiserum from the colostrum of mammals is well-known for the man skilled in the art and also the technique of affinity purification of anti-sera is commonly accepted in the routine experimentation of immunologists.

V.2.6. The subject-matter of the claims 5-9 appears to be novel and inventive (Art. 33 (1)-(3) PCT), since in view of the documents cited in the International Search Report there is neither a disclosure nor an incentive to disclose a method of treating a mammal prophylactically to prevent neoplastic development as claimed in claim 5, a pharmaceutical formulation for use in the therapy of neoplastic conditions containing venom in combination with antiserum to Phospholipase C enzyme (claim 6) and the corresponding method (claim 22) or a pharmaceutical formulation containing one or more venoms as antigen in combination with Phospholipase C enzyme (claim 8). Furthermore, the subject-matter of claim 18 seems to be novel and inventive, since Schizophrenia has not been disclosed or suggested as being cured by administration of antiserum to venom which is reactive with at least one PLA_2 enzyme according to claim 1. Also a method of inoculation of human or animal with a combination of two or more phospholipase A_2 enzyme types (claim 26) appears to be novel and inventive with respect to the documents cited in the International Search Report (but see *Section VIII.*3.).

V.3. *Industrial applicability:*
For the assessment of the present claims 1-5, 10-19, 21-42 on the question whether

they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims.
The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Section VIII:

VIII.1. The subject-matter of the claims 1 and 6 is not clear (Art. 6 PCT), since according to the description (page 1, line 25; page 2, line 25) the antiserum to venom, mammalian, plant or insect tissue demonstrating the ability to bind Phospholipase A_2 (PLA_2) enzymes is shown to be active as anti-proliferative neoplastic agent.
However, according to the present wording of the claims 1 and 6 , also the venom itself could be meant.

VIII.2. Claim 7 refers to the method according to claim 6. However, the subject-matter of claim 6 defines no method, but a product, namely a pharmaceutical formulation.
The same applies to claim 9 referring to claim 8 and to the claims 10-17, 19, 21-25, 32 as far as they are dependant on the claims 6 and 8, which are product-claims and do not define a method.
Conversely, claim 20 defines a product, namely therapeutic agents, and refers to the claims 1 and 5, which do not characterize products, but methods.
Furthermore, the subject-matter of the claims 23-25 defines the antiserum. However, these claims refer to the claims 5 and 8, which do not contain any antiserum, but the antigen. Therefore, these claims are not clear.

VIII.3. Claim 26 does not comply with the requirements of Art. 6 PCT, because it is formulated as an independent claim but contains all the features of claim 5.

VIII.4. The subject-matter of the claims 33-37 is not disclosed in the description in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art (Art. 5 PCT).

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. 09/254623

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* F8009-7004

Box No. I TITLE OF INVENTION

Therapeutic Formulations Containing Venom or Venom Anti-Serum Either Alone or in Combination for the Therapeutic Prophylaxis and Therapy of Neoplasms

Box No. II APPLICANT

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☒ This person is also inventor.

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This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

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This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☐ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BROWN, Kevin C.
Nikaido, Marmelstein, Murray & Oram LLP
655 15th Street N.W. Suite 330
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Washington, DC 20005-5701
US

Telephone No.
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(202) 638-4810

Teleprinter No.

☒ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ **AP ARIPO Patent:** **GH** Ghana, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ **AL** Albania .....
☒ **AM** Armenia .....
☒ **AT** Austria .....
☒ **AU** Australia .....
☒ **AZ** Azerbaijan
☒ **BA** Bosnia and Herzegovina .....
☒ **BB** Barbados
☒ **BG** Bulgaria .....
☒ **BR** Brazil .....
☒ **BY** Belarus
☒ **CA** Canada
☒ **CH and LI** Switzerland and Liechtenstein
☒ **CN** China .....
☒ **CU** Cuba .....
☒ **CZ** Czech Republic .....
☒ **DE** Germany .....
☒ **DK** Denmark .....
☒ **EE** Estonia .....
☒ **ES** Spain .....
☒ **FI** Finland .....
☒ **GB** United Kingdom
☒ **GE** Georgia .....
☒ **GH** Ghana .....
☒ **HU** Hungary .....
☒ **IL** Israel .....
☒ **IS** Iceland
☒ **JP** Japan .....
☒ **KE** Kenya .....
☒ **KG** Kyrgyzstan .....
☒ **KP** Democratic People's Republic of Korea .....
.....
☒ **KR** Republic of Korea .....
☒ **KZ** Kazakstan .....
☒ **LC** Saint Lucia
☒ **LK** Sri Lanka
☒ **LR** Liberia
☒ **LS** Lesotho .....
☒ **LT** Lithuania
☒ **LU** Luxembourg .....
☒ **LV** Latvia
☒ **MD** Republic of Moldova .....
☒ **MG** Madagascar .....
☒ **MK** The former Yugoslav Republic of Macedonia
.....
☒ **MN** Mongolia
☒ **MW** Malawi .....
☒ **MX** Mexico .....
☒ **NO** Norway
☒ **NZ** New Zealand .....
☒ **PL** Poland .....
☒ **PT** Portugal .....
☒ **RO** Romania
☒ **RU** Russian Federation .....
☒ **SD** Sudan
☒ **SE** Sweden
☒ **SG** Singapore
☒ **SI** Slovenia .....
☒ **SK** Slovakia .....
☒ **SL** Sierra Leone
☒ **TJ** Tajikistan .....
☒ **TM** Turkmenistan .....
☒ **TR** Turkey .....
☒ **TT** Trinidad and Tobago .....
☒ **UA** Ukraine .....
☒ **UG** Uganda .....
☒ **US** United States of America .....
.....
☒ **UZ** Uzbekistan .....
☒ **VN** Viet Nam .....
☒ **YU** Yugoslavia .....
☒ **ZW** Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☒ ID . . Indonesia. .....
☐ .....
☐ .....
☐ .....
☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
Item (1) US	11/September/1996 (11-09-96)	60/025,179	
Item (2)	( )		
Item (3)	( )		

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of this present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA/EP

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	3 sheets
2. description	:	30 sheets
3. claims	:	4 sheets
4. abstract	:	2 sheets
5. drawings	:	sheets
Total	:	39 sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify):*

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

xElizabeth Shanahan-Prendergast

Dr. Elizabeth Shanahan-Prendergast

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference F8009-7004

Applicant

Elizabeth SHANAHAN-PRENDERGAST

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 200.00 T

2. SEARCH FEE . . . . . 1,310.00 S

International search to be carried out by EP

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 39 sheets.

first 30 sheets . . . . . 530.00 b_1

9 (remaining sheets) x 10.00 (additional amount) = 90.00 b_2

Add amounts entered at b_1 and b_2 and enter total at B . . . . 620.00 B

Designation Fees

The international application contains 73 designations.

11 (number of designation fees payable *(maximum 11)*) x 128.00 (amount of designation fee) = 1,408.00 D

Add amounts entered at B and D and enter total at I . . . . . 2,028.00 I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT . . . . . P

5. TOTAL FEES PAYABLE

Add amounts entered at T, S, I and P, and enter total in the TOTAL box . . 3,538.00 TOTAL

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☐ authorization to charge deposit account (see below)	☐ bank draft	☐ coupons
☒ cheque	☐ cash	☐ other *(specify)*:
☐ postal money order	☐ revenue stamps	

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ _____

☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☐ is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account Number _____ Date *(day/month/year)* _____ Signature _____

See Notes to the fee calculation sheet

19

REC'D 17 DEC 1998

WIPO PCT

PATENT COOPERATION TREATY

PCT

09/254623

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
F8009-7004		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB97/01091	10/09/1997	11/09/1996
International Patent Classification (IPC) or national classification and IPC		
A61K35/58		
Applicant		
SHANAHAN-PRENDERGAST, Elizabeth		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 28 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/03/1998	15.12.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Thalmair, M Telephone No. (+49-89) 2399-2177

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB97/01091

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-15	as received on	23/01/1998	with letter of	23/01/1998

Claims, No.:

1-42	as received on	23/01/1998	with letter of	23/01/1998

Drawings, sheets:

1/9-9/9	as received on	23/01/1998	with letter of	23/01/1998

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13.15.18-19.22-32.34.37-42
	No:	Claims	14.16.17.20,21.33.35.36
Inventive step (IS)	Yes:	Claims	5-9.18.22.26-28
	No:	Claims	1-4.10-17.19.20-21.23-25.29-42
Industrial applicability (IA)	Yes:	Claims	1-42
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

The documents which are referred to in this communication are numbered in the same order as cited in the International Search Report.

Section V:

V.1. *Novelty:*

V.1.1. The subject-matter of the claims 14, 16, 17, 20, 21 is not novel (Art. 33 (1) and (2) PCT) for the following reasons.

D 5 already discloses monoclonal antibodies (mAb) and fragments thereof to type I Phospholipase A_2 (PLA_2) for the treatment of inflammatory reactions, e.g. acute pancreatis (c.f. claims 14, 17, 20, 21).

D 6 shows the use of inhibitors of the function of murine and human PLA_2 such as antibodies to PLA_2 for the therapeutic treatment of inflammatory reactions, e.g. rheumatoid arthritis (c.f. claim 20 of the present application), psoriasis (claims 16, 20), asthma (claims 16, 20).

V.1.2. The subject-matter of the claims 33 and 35 is not novel (Art. 33 (1) and (2) PCT), since D 4 discloses the use of mAb recognizing membrane PLA_2 for the diagnosis of cancer via the measurement of PLA_2 level (see column 2, lines 1-32). Moreover, the subject-matter of claim 36 is not novel, since D 1 discloses PLA_2 and fragments thereof from snake venom used for detection of tumoural cells (see column 5, lines 29 ff.).

V.2. *Inventive Step:*

V.2.1. The subject-matter of the claims 1-4, 15, 23, 24, 39 does not involve an inventive step according to Art. 33 (1) and (3) PCT for the following reasons.

D 4 shows the use of mAb to human membrane PLA_2 for the diagnosis of cancer.

D 6 discloses the use of inhibitors of the function of murine and human PLA_2 such as antibodies to PLA_2 for the treatment of inflammatory reactions, but also of other diseases mediated e.g. by prostaglandins (see column 15, line 16).

Prostaglandins like PGE_2 are known inhibitors of cell mediated immunity which have an effect on cell proliferation in tumour growth by decreasing tumour cell killing according to the description of the present application (page 4, line 10- to page 5, first paragraph). Therefore, the man skilled in the art would certainly derive from D 6 in combination with D 4, that antiserum to PLA_2 derived from mammalian or other origin (claim 3) is useful in the treatment of neoplasm in a mammal (claim 1).

The same applies to the subject-matter of claim 15.
Moreover, it is well-known in the art that anti-serum (especially polyclonal) often reacts with more than one types of one enzyme (see claims 2, 39).
Furthermore, the man skilled in the art would expect to be successful in using either polyclonal or monoclonal antiserum (claims 4, 23) or antiserum generated in eggs, since the technique of generating antibodies in eggs is widely accepted in the art of immunology (claim 24).

V.2.2. The subject-matter of the claims 10 and 11 is not inventive (Art. 33 (1) and (3) PCT), since the man skilled in the art would certainly try to use the claimed therapeutic agents combined with other therapeutically effective agents such as other known antiinflammatory/immunosuppressive or chemotherapeutic agents (see page 7 of the description) and expect to be successful especially as there is no surprising effect recognizable from the description. The combination with adjuvants is obvious since the combining of an active agent with adjuvants is commonly employed in the state of the art.
The use of venom originating from snake according to claim 12 is derivable for instance from D 1, D 2, D 3 and does not involve an inventive step.
Furthermore, as it is known that venom (for instance cobra venom) contains PLA_2 and that for instance mammalia including humans contain these lipolytic enzymes (see page 2 of the present application), the subject-matter of the claims 30 and 31 is obvious for the skilled person.
The use of more than one species of snake according to claim 13 is also not inventive, since D 1 already shows a basic protein, namely a PLA_2 isolated from snakes, namely from Naja nigricollis and / or from Naja mossambica pallida (see abstract).

V.2.3. The subject-matter of the claims 19 and 32 is not inventive (Art. 33 (1) and (3) PCT) , since the synthetic productions of anti-sera and of enzymes are well-known techniques in the art, see for instance D 6, which mentions that PLA_2 enzymes can be purified from cell sources or produced recombinantly or synthetically (see column 14, lines 35-37).

V.2.4. Even if the subject-matter of claim 20 is rendered novel, it would not involve an inventive step according to Art. 33 (1) and (3) PCT with respect to the treatment of all diseases which are linked to inflammatory processes such as rheumatoid arthritis,

osteoarthritis, gout, rheumatic carditis, allergic diseases, ocular and dermal inflammatory diseases, acne, allergic conjunctivitis, Graves disease, Lupus, Reiter-Krankheit, since the man skilled in the art regarding D 6 would easily find out the special kinds of inflammatory diseases for which the claimed therapeutic agents are best suited.
Also the subject-matter of the claims 38, 41, 42 does not involve an inventive step, since the skilled man in the art would consider D 6, which discloses the use of inhibitors of the function of murine and human PLA_2 such as antibodies to PLA_2 for the treatment of inflammatory reactions, and derive that these therapeutic agents of D 6 would also be useful in the treatment of inflammatory reactions resulting from parasitic and bacterial infections.

V.2.5. The subject-matter of claim 25 does not include an inventive step, since the extraction of antiserum from the colostrum of mammals is well-known for the man skilled in the art and also the technique of affinity purification of anti-sera is commonly accepted in the routine experimentation of immunologists.

V.2.6. The subject-matter of the claims 5-9 appears to be novel and inventive (Art. 33 (1)-(3) PCT), since in view of the documents cited in the International Search Report there is neither a disclosure nor an incentive to disclose a method of treating a mammal prophylactically to prevent neoplastic development as claimed in claim 5, a pharmaceutical formulation for use in the therapy of neoplastic conditions containing venom in combination with antiserum to Phospholipase C enzyme (claim 6) and the corresponding method (claim 22) or a pharmaceutical formulation containing one or more venoms as antigen in combination with Phospholipase C enzyme (claim 8). Furthermore, the subject-matter of claim 18 seems to be novel and inventive, since Schizophrenia has not been disclosed or suggested as being cured by administration of antiserum to venom which is reactive with at least one PLA_2 enzyme according to claim 1. Also a method of inoculation of human or animal with a combination of two or more phospholipase A_2 enzyme types (claim 26) appears to be novel and inventive with respect to the documents cited in the International Search Report (but see *Section VIII.*3.).

V.3. *Industrial applicability:*
For the assessment of the present claims 1-5, 10-19, 21-42 on the question whether

they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims.
The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Section VIII:

VIII.1. The subject-matter of the claims 1 and 6 is not clear (Art. 6 PCT), since according to the description (page 1, line 25; page 2, line 25) the antiserum to venom, mammalian, plant or insect tissue demonstrating the ability to bind Phospholipase A_2 (PLA_2) enzymes is shown to be active as anti-proliferative neoplastic agent.
However, according to the present wording of the claims 1 and 6 , also the venom itself could be meant.

VIII.2. Claim 7 refers to the method according to claim 6. However, the subject-matter of claim 6 defines no method, but a product, namely a pharmaceutical formulation.
The same applies to claim 9 referring to claim 8 and to the claims 10-17, 19, 21-25, 32 as far as they are dependant on the claims 6 and 8, which are product-claims and do not define a method.
Conversely, claim 20 defines a product, namely therapeutic agents, and refers to the claims 1 and 5, which do not characterize products, but methods.
Furthermore, the subject-matter of the claims 23-25 defines the antiserum. However, these claims refer to the claims 5 and 8, which do not contain any antiserum, but the antigen. Therefore, these claims are not clear.

VIII.3. Claim 26 does not comply with the requirements of Art. 6 PCT, because it is formulated as an independent claim but contains all the features of claim 5.

VIII.4. The subject-matter of the claims 33-37 is not disclosed in the description in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art (Art. 5 PCT).

PATENT COOPERATION TREATY

PCT

09/254623

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
F8009-7004		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IB 97/01091	10/09/1997	11/09/1996
Applicant		
SHANAHAN-PRENDERGAST, ELIZABETH		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [ ] the text is approved as submitted by the applicant.
   - [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PCT/[redacted] 97/01091

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/58 A61K39/395 A61K38/46 G01N33/574 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 322 262 A (COMMISSARIAT À L'ÉNERGIE ATOMIQUE) 28 June 1989 see column 5, line 11 - line 15 see column 5, line 20 - line 47 see claims 12-15 ---	1-5, 10-12, 20,32
X	US 5 164 196 A (PLATA ET AL.) 17 November 1992 see the whole document ---	1,5,10, 12,20
X	EP 0 246 861 A (PLATA ET AL.) 25 November 1987 see the whole document ---	1,5,10, 12,14,20
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search
9 December 1997

Date of mailing of the international search report

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 459 450 A (SHIONOGI SEIYAKU KK) 4 December 1991 see the whole document	1,3,4, 14,17, 20,23,33
X	DE 41 42 552 A (BOEHRINGER MANNHEIM GMBH) 24 June 1993 see the whole document	1,3,4, 10,11, 14,17, 20,21,23
X	US 5 322 776 A (KNOPF ET AL.) 21 June 1994 see column 14, line 67 - column 15, line 43	1,16,17, 20,23, 32,35
P,X	US 5 565 431 A (LIPPS ET AL.) 15 October 1996 see the whole document	1,5,10, 12,13,20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 97/01091

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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Claims 7,9-19,21-25 and 32 represent an obscurity, since, as a method claim, they, completely or partially, refer to one or more product claims.

Remark : Although claims 1-5,7,9-19,21-32,35, and 38-42 (all completely), and claims 36 and 37 (partially, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body, and although claims 33 and 34 (both partially, as far as an in vivo method is concerned) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Box III TEXT OF THE ABSTRACT (Continuation of Item 5 of the first sheet)

The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom and/or other components of animal, insect or plant origin showing Phospholipase A_2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial, Phospholipase A_2 enzyme activity alone or in combination with animal or plant Phospholipase A_2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono or polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the anti-serum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA_2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial, Phospholipase A_2 and C enzyme activity.

INTERNATIONAL SEARCH REPORT

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(54) Title: THERAPEUTIC FORMULATIONS CONTAINING VENOM OR VENOM ANTI-SERUM EITHER ALONE OR IN COMBINATION FOR THE THERAPEUTIC PROPHYLAXIS AND THERAPY OF NEOPLASMS

(57) Abstract

The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom and/or other components of animal, insect or plant origin showing Phospholipase A_2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial, Phospholipase A_2 enzyme activity alone or in combination with animal or plant Phospholipase A_2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono- or polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the anti-serum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA_2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial, Phospholipase A_2 and C enzyme activity.

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THERAPEUTIC FORMULATIONS CONTAINING VENOM OR VENOM ANTI-SERUM EITHER ALONE OR IN COMBINATION FOR THE THERAPEUTIC PROPHYLAXIS AND THERAPY OF NEOPLASMS

The present invention comprises the method of treating a host organisms (man or animal) in need of a drug having direct or prophylactic anti-neoplastic activity comprising the administration of a therapeutically effective amount of Phospholipase A_2 targeted venom anti-serum alone or in combination with a known Phospholipase C anti-serum or a Phospholipase C inhibitory compound. A vaccine containing in whole or in part snake or insect venom or mammalian PLA_2 components comprising epitopes demonstrating Phospholipase A_2 activity and/or Phospholipase C enzyme components. This patent presents therapeutic pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which contain, total or partial, phospholipase A_2 enzyme activity or PLA_2 epitopes. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms and/or mammalian PLA_2 enzymes wherein the anti-serum has been preferably affinity purified for use in treating patients suffering from neoplastic disease. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect venoms or the PLA_2 enzyme components thereof and/or PLA_2 enzymes isolated from insect, mammalian on plant cells, and/or Phospholipase C enzyme preparation or extract from such venoms which may contain, total or partial, phospholipase A_2 enzyme activity.

In this patent the affinity purified anti-serum to venoms Phospholipase A_2 (PLA_2) and mammalian or plant PLA_2 are shown to be active anti - proliferative neoplastic agents.

The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom

and/or other components of animal, insect or plant origin showing Phospholipase A_2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial, Phospholipase A_2 enzyme activity alone or in combination with animal or plant Phospholipase A_2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono or polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the antiserum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA_2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial, Phospholipase A_2 and C enzyme activity.

Phospholipase A_2 are lipolytic enzymes that hydrolyze the sn-2-acylester bond in glycerophospholipids. Many forms of PLA_2 exist in nature and have been described and classified into several groups. Types I, II and III PLA_2 are low molecular weight peptides (13-18 kDa) extra-cellular enzymes, including pancreatic and cobra venom PLA_2 (type I), rattle snake and inflammatory PLA_2 (type II) and bee venom type III. Intracellular cytosolic PLA_2 belong to different groups, including the 85 kDa (type IV) and 40-75 kDa enzymes.

Affinity purified anti-serum to venoms, animal or plant tissue demonstrating the ability to bind PLA_2 enzymes are shown herein below, by way of example, to be active in-vitro and in-vivo anti-proliferative neoplastic agents. Accordingly, these affinity purified antisera either alone or in combination with non-toxic Phospholipase C inhibitor or anti-serum to Phospholipase C are useful in the control of proliferation of neoplastic tissue.

BACKGROUND OF THE INVENTION

There is evidence to indicate that Phospholipase A_2 (PLA_2) is involved in the pathogenesis of many diseases. Thus local and circulating levels of Phospholipase A_2 enzyme and enzymatic products are elevated during infection, inflammatory diseases, tissue injury and brain dysfunction and is a very early indication of neoplastic development prior to tumour cell mass being evident by conventional methods of scanning tissue tumours.

Excessive Phospholipase A_2 activity may promote chronic inflammation, allergic reaction, tissue damage and pathophysiological complications. These effects may be the result of accumulating Phospholipase A_2 products (lysophospholipids and free fatty acids, e.g. Arachidonic Acid) and destruction of key structural phospholipid membrane components, but are potentated by secondary metabolites, such as eicosanoids and platelet-activating factor. Phospholipase A_2 products or lipid mediators derived therefrom have been implicated in numerous activities that are an integral part of cell activation; chemotaxis, adhesion, degranulation, phagocytosis and aggregation.

Phospholipase A_2 secreted excessively at local sites may be responsible for tissue damage common to rheumatic disorders, alveolar epithelial injury of lung disease and reperfusion.

During acute myocardial ischemia, cytosolic Phospholipase A_2 and Phospholipase C activation causes increased intracellular Ca^{2+}. Subsequent accumulation of lysophospholipids and free fatty acids promote damage to sarcolemmal membranes leading to irreversible cell injury and eventually cell death.

Altered cytosolic Phospholipase A_2 and Phospholipase C activity or defects in their control and regulation is a predisposing factor to causing tumour cell development.

Prostaglandins and related eicosanoids are important mediators and regulators of both immune and inflammatory responses. Prostaglandin E_2 induces bone resorption and Leukotriene B_4 stimulates vascodilation and chernotaxis. Increased levels of Phospholipase A_2 is noted in Rheumatoid Arthritis (R.A.), osteoarthritis, gout, collagen and vascular diseases.

Phospholipase A_2 induces non specific airway hyperactivity that is evident in asthma. Phospholipase A_2 is also elevated in peritonitis, septic shock, renal failure, pancreatis, Chrons and Graves Disease.

The activity of cell-mediated defence systems is stimulated by consecutive formation of interleukin 1β(IL-1β), interleukin-2 (IL-2) and interferon γ (IFN γ). The system is inhibited by interleukin-4 (IL-4) and also by prostaglandin E_2 (PGE_2) and histamine, which are released when the immune system is activated. The inhibition is strong in cancer patients, because PGE_2 is formed in many cancer cells and its formation is stimulated by IL-1β. PGE_2 and histamine are feedback inhibitors of cell mediated immunity.

PGE_2 is formed from arachidonic acid in monocytes, macrophages, cancer cells and other cells, when arachidonic acid is released from cellular phospholipids. The formation of PGE_2 is stimulated by several compounds, including histamine, IL-1 (α and β) and Tumour Necrosis Factor α (TNFα). PGE_2 inhibits the formation and receptor expression of IL-2 by increasing the level of cyclic AMP (cAMP) in helper T cells. This concomitantly decreases the formation of IFN γ.

PGE_2 inhibits the ability of natural killer cells (NK) to bind with tumour cells by increasing cAMP in Natural Killer Cells. This decreases tumour cell killing.

When the immune system is stimulated to destroy tumour cells, the killing is prevented because IL-1β stimulates PGE_2 formation in tumour cells, which increases cAMP levels in NK cells and prevents the binding of NK and tumour cells.

The activation of the cell-mediated defence is blocked also because PGE_2 -increases cAMP in helper T cells and inhibits the formation of IL-2 and IFNγ.

Cytotoxic T cells can also produce PGE_2 thus inhibiting the activity of NK cells.

A number of human and experimental animal tumours, contain and/or produce large quantities of prostaglandins (PG). Prostaglandins E_2 has been shown to effect significantly cell proliferation in tumour growth and to suppress immune responsiveness.

Phosphatidylinositol specific phospholipase C is an important enzyme for intracellular signalling. There are at least three major classes of Phosphatidylinositol specific Phospholipase C (PtdInsPLC: PtdInsPLC β, γ, δ). PtdInsPLCs hydrolyse a minor membrane phospholipid, phosphatidylinositol (4, 5) bisphosphate (PtdIns (4,5) P_2) to give the second messengers inositol (1, 4, 5) trisphosphate (Ins (1, 4, 5) P_3), which releases Ca++ from intracellular stores to increase the intracellular free CA++ concentration, and diacylglycerol which activates the Ca++ and phospholipid-dependent protein serine/threonine kinase, protein kinase C. Proteins phosphorylated by protein kinase C include transcription factors. Together, the increase in intracellular free Ca++ concentration and the activation of protein kinase C lead to a series of events that culminate in DNA synthesis and cell proliferation in tumour cells.

A number of growth factors and mitogens, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and bombesin, act through specific receptors to increase Ptd Ins PLC activity in cells. Continued stimulation of Ptd Ins PLC can lead to cell transformation.

Ptd Ins PLC activity is found to be increased in a number of human tumours. 76% of human breast cancers have detectable Ptd Ins PLC-γ immunoreactive protein compared to only 6% in benign breast tissue.

Cytosolic Ptd Ins PLC activity is increased up to >4-fold in human non-small cell lung cancer and renal cell cancer compared to normal tissue.

SUMMARY OF THE INVENTION

The present invention comprises the method of treating mammals including humans in need of a drug to prevent neoplastic tissue growth and spread by the administration of a therapeutically effective amount of venom anti-serum prepared to whole venom or to parts of the venom or components

of plant or animal origin which demonstrate PLA_2 activity. Also enhanced anti-cancer effects both in-vitro and in-vivo have been realised by combining this affinity purified anti-serum to PLA_2 components and/or mammalian PLA_2 with a non-toxic inhibitor of Phospholipase C or with anti-serum (polyclonal or monoclonal) developed to Phospholipase C enzyme.

This patent relates to the administration of one or more compounds which can generally be described as performing their fimction by either directly or indirectly causing Phospholipase A_2 and/or Phospholipase C enzyme inhibition, wherein the said inhibition is either partial or total. In addition this patent relates to the administration of one or more compounds which can generally be described as performing their function by interaction with the neoplastic cell membrane preventing their growth or spread, thus preventing further disruption of non-involved organs of the body and causing no toxicity to the infected patient or animal being treated.

Additional aspects of the invention relates to pharmaceutical compositions containing the compounds of the invention as active ingredients, modifying unwanted immune responses, and to methods of retarding proliferation of tumour cells using the compounds and compositions of this invention.

The anti-serum to snake venom PLA_2 and to plant, insect, mammalian and/or to PLA_2 epitopes or mimic molecules are shown herein to be active anti-tumour proliferative compounds and immune enhancing. For use in this regard, the compounds of the invention are administered to mammals, including humans, in an effective amount of 0.05 to 5 gms per day per kilogram of body weight. The amount depends, of course, on the condition to be treated, the severity of the condition, the route of administration of the drug, and the nature of the subject. The drugs may be administered IV, orally, parenterally, or by other standard administration routes including targeting with liposomes/RBC.

The therapeutic activity of the compounds of this invention are demonstrated by inhibition of the tumour cell lines in-vitro and in-vivo. The

compounds were tested for toxicity in Scid mice. Results as in Figure 1 [toxicity data].

Toxicity Study

Method

Female Scid mice (6-8 weeks of age) were treated with either a Neat or a 1:10 dilution of the anti-serum preparation, subcutaneously (0.1 ml, daily) for a period of 14 days. The weights of the mice were measured daily. At termination, organs were removed and fixed in formalin for histological examination.

Results

No toxicity, as assessed by animal weights and clinical well-being, was evident (Figure 1).

The compounds of this invention may be combined with other known anti-inflammatory/immunosuppressive or chemotherapeutic agents such as steroids or non-steroidal anti-inflammatory agents in the pharmaceutical compositions and methods described herein.

Anti-serum to snake and/or insect venoms and/or mammalian and/or PLA_2 enzyme or its epitopes can be used as a therapeutic treatment in diseases where elevated levels of Phospholipase A_2 are evident, (e.g. Rheumatoid Arthritis, see Table B). It is also envisaged that this novel therapy with anti-serum to venom PLA_2 (snake or insect) and/or to PLA_2 components (derived from animal or plant) can be applied as a prophylactic therapy by using sub-lethal doses of venoms or the venom PLA_2 enzyme extracts together with mammalian or plant PLA_2 or synthetic peptides demonstrating PLA_2 activity plus adjuvant to stimulate an immunoglobulin response within the patient, see results - Vaccine Efficacy in Balb/c mice. It is also envisaged that a synthetic peptide incorporating the Phospholipase A_2 and/or Phospholipase C activity could be used to generate said anti-serum or therapeutic agent or vaccine. Use may also be made in the generating of this therapeutic vaccine/anti-serum by using the known sequence homology that exists between human Phospholipase A_2 and snake/insect venoms

together with animal PLA_2 used in combination with compounds known to inhibit Phospholipase C activity or anti-serum developed to this enzyme.

Sustained or directed release compositions can be formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable coatings, e.g. by microencapsulation, multiple coatings, etc.. It is also possible to freeze-dry the new compounds and use the lyophilizates obtained, for example, for the preparation of products for storage and subsequent injection.

EXPERIMENTATION

The compounds of this invention can be identified as anti-serum to snake or insect venoms mammalian or plant PLA_2 or parts thereof or Phospholipase C or mimic molecules generated to venoms or mammalian PLA_2 molecules and/or Phospholipase C or parts thereof also the pharmaceutical use of venoms or parts thereof and/or mammalian PLA_2 or enzyme components as vaccine antigen are incorporated. Non-toxic compounds showing anti-phospholipase C activity can be incorporated with the anti-serum to PLA_2 of any origin, or mimic molecules demonstrating Phospholipase A_2 activity.

In certain applications of this therapy it may be necessary to curtail the ADCC reaction which could cause serum sickness and to ensure that this does not occur the IgG (FC) component is enzymatically cleaved from the affinity purified immunoglobulin so that natural killer cells will not react to the immunoglobulin in the anti-serum.

Ability of anti-serum to snake venom to inhibit Phospholipase A_2 enzyme isolated from human synovial fluid (Table A2).

The inhibition of Phospholipase A_2 enzyme from synovial fluid isolated from a patient with Rheumatoid Arthritis was tested with a range of dilutions of anti-serum to snake venom. Anti-serum to snake venom generated in horse, reconstituted in 10 ml sterile water. The following dilutions were used 1:10, 1:20, 1:40 and 1:60. The method used was as outlined in "Infection and

Immunity, Sept. 1992, p. 3928-3931. Induction of Circulating Group II Phospholipase A_2 Expression in Adults with Malaria.

Results (Table A2)

Dilution	Inhibition
1:10	63%
1:20	50%
1:40	35%
1:60	29%

In-vitro testing of un-affmity purified snake venom.

A range of tumour cell lines were tested with 3 concentrations of the anti-serum to snake venom by the MTT Assay. This anti-serum was not affinity purified. MTT Assay described by Alley et al, (Cancer Research, 48: 589-601, 1988) See Table B.

SUMMARY OF RESULTS (Table B)

Molt 4:	**Human T cell Lymphoma Cancer**
Serum-containing	
Dilution	**% of Control**
Neat	48.1
1:10	53.7
1:20	40.8
Serum-Free	
Neat	58.7
1:10	51.2
1:20	40.6
MDA 468:	**Human Breast Cancer**
Serum-containing	
Dilution	**% of Control**
Neat	8.0
1:10	53.7
1:20	58.9
Serum-Free	

Neat	15.4
1:10	48.4
1:20	58.9

C17OHM2: Human Colon Cancer

Serum-containing

Dilution	**% of Control**
Neat	9.3
1:10	61.4
1:20	55.6

Serum-Free

Neat	15.2
1:10	47.3
1:20	49.5

Pan 1: Human Pancreatic Cancer

Serum-Containing

Dilution	**% of Control**
Neat	9.3
1:10	47.5
1:20	49.2

Serum-Free

Neat	43.1
1:10	53.2
1:20	69.4

841: Human small cell lung cancer

Serum-containing

Dilution	**% of Control**
Neat	25.2
1:10	45.5
1:20	51.1

Serum-Free

Neat	63.4

1:10	60.1
1:20	59.8

T24: Human Bladder Cancer

Serum-containing

Dilution	**% of Control**
Neat	68.5
1:10	75.1
1:20	76.2

Serum-Free

Neat	84.1
1:10	87.9
1:20	88.4

Testing un-affinity purified anti-serum to Snake Venom against B16F1 Melanoma Cell Line.

Mice

C57BL/6

Procedure

The mice were inoculated with 0.5 $x10^6$ B16 F1 melanoma cells subcutaneously (sc) into flank region. Once palpable tumours had developed the mice received daily sc injections as follows: -

			number of mice
A	-	Sterile water 100µl	6
B	-	anti-serum (full strength) 100µl	6
C	-	anti-serum (diluted 1:10) 100µl	6

The dimensions of the tumours were taken daily using callipers. Once the tumours of the control mice were approximately 1.5 cm or larger in diameter all mice were killed. The tumours were removed and weighed.

Results

Small tumours were first discernible by palpitation in all mice 6-7 days after inoculation. The changes in volume as measured by callipers, together with

tumour weights at autopsy. See Fig. 2 [Effect of un-affinity purified anti-serum to snake venom on Melanoma B16F1 Growth] for effect of anti-serum to snake venom on tumour growth retardation.

IN-VITRO SREENING OF THE AFFINITY PURIFIED ANTI-SERUM TO SNAKE VENOM PREPARATION AGAINST A RANGE OF TUMOUR CELL LINES (Illustrated in Fig. 3A [Human colorectal tumour C170HM2], Fig. 3B [Human bladder tumour T24], Fig. 3C [Human lymphoma tumour MOLT 4], Fig. 3D [Human pancreatic tumour PAN 1], Fig. 3E [Human breast tumour MDA 468], Fig. 3F [Human small cell lung tumour 841], Fig. 3G [Human gastric ST24], and Fig. 3H [Human Ovarian OVCAR3])

Introduction

The in-vitro inhibitory effects of the horse generated anti-serum to snake venom preparation, previously evaluated were obscured due to serum enhancement of tumour cell growth. Thus in the following assay, affinity purified anti-serum to snake venom was evaluated.

Method

The cell lines were seeded into 96 well plates at a cell concentration of 10^4 cells per well in both serum free (Hams F12:RPMI 1640 + 0.5% bovine serum albumen) and serum-containing medium (RPMI 1640 + 10% heat inactivated foetal calf serum). The anti-serum preparation was diluted in the corresponding medium and added to the wells, 2-3 hours after the cells (to allow for cell adherence). The plates were incubated at 37 °C in -5% $C0_2$ for 3 days. The cells were then incubated with 1 mg/ml MTT (methyl thiazol tetrazolium) for 4 hours at 37 °C. The crystals were then solublised with dimethyl sulphoxide and the absorbance measured at 550nm.

Results

The test anti-sera inhibited all of the cell lines at all concentrations examined.

The level of inhibition was statistically significant from the untreated control at all anti-serum dilutions, with all cell lines as assessed by a one way analysis of variance.

IN-VIVO TEST

The effects of affinity purified anti-serum to snake venom on human colorectal C17OHM$_2$ cell line.

Materials and Methods

C170MH$_2$ cells were injected subcutaneously into the left flank of ten male nude mice. The mice were allocated randomly to two groups.

Group 1 - 100µl anti-serum twice daily intravenously (IV)

Group 2 - 100µl PBS twice daily IV

Tumours were measured twice weekly, using callipers, in two dimensions. Cross-sectional areas were calculated. The mice were also weighed once weekly. The therapy was terminated at day 22.

Results

The cross-sectional areas were measured at increasing time points during the experiment, as shown in Fig. 4 [Effect of affinity purified anti-serum to snake venom on the mean cross-sectional area of C170HM2 in nude mice]. The affinity purified anti-serum preparation induced a slowing in growth when compared to saline controls. An ANOVA was performed on the results in which the treatment was evaluated with respect to time, and shows a significance of P = 0.028.

At the termination of the experiment, the tumours were weighed and the results are shown in Fig. 5 [Effect of affinity purified anti-serum to snake venom on the final tumour weight of C170HM2]. No toxic effect of the affinity purified anti-serum preparation was observed.

In-vitro screen of the affinity purified anti-serum to snake venom preparation in combination with a phospholipase C inhibitor 1-oleoyl-2-acetyl-sn-glycerol (OAG) 5µ molar, on a range of cancer cell lines.

Methods

The affinity purified anti-serum to snake venom preparation was diluted 1:2 and 1:10 and was combined with 5 µ molar OAG and added to the wells as previously described for the MTT Assay. The cell lines tested were Human Breast tumour, MDA 468, Human small cell lung tumour 841 and

Human renal TK-10. Results as shown in Fig. 6A [Affinity purified anti-serum to snake venom and (OAG) a Phospholipase C inhibitor combination--Human breast tumour MDA 468], Fig. 6B [Affinity purified anti-serum to snake venom and (OAG) a phospholipase C inhibitor combination--Human small cell lung tumour 841] and 6C [Affinity purified anti-serum to snake venom and (OAG) a phospholipase C inhibitor combination--Human renal TK-10].

In-vivo testing of the combination of affinity purified anti-serum to snake venom and 1-oleoyl-2-acetyl-sn-glyceral (OAG) at 5µm concentration on the growth of MDA 468 cell line.

Method

MDA 468 tumours were aseptically removed from donor female Scid mice. The tissue was aseptically minced, pooled and implanted into anaesthetised female Scid mice (anaesthetic comprised of a 0.2 ml injection of Hypnorm (Jannsen) :Hyonovel (Roche) : distilled water in a 1: 1:5 ratio). Tissue implants consisted of 3-5 mm^2 pieces and after subcutaneous transplantation into the left flank, the incision was clipped. The Scid mice were then randomised into 2 groups of 10 animals. They were treated daily with a 0.2 ml subcutaneous injection (in the opposite flank to the tumour graft) of a combination of affinity purified anti-serum to snake venom and 5µm molar of (OAG) dilution of the anti-serum preparation. The control animals received 0.2 ml phosphate buffered saline, pH 7.6. All animals were terminated on day 63, and the tumours were dissected out, weighed and processed for histology. Results are in Fig. 7 [Effect of the affinity purified anti-serum to venom in combination with the Phospholipase C inhibitor (OAG) 5 µm].

Vaccine Efficacy in Balb/c mice after challenge with WEHI-3 cell.

The objective of study is to demonstrate the efficacy of sub-lethal levels of Russelli vipera venom entrapped in liposomes and porcine phospholipase A_2 enzyme entrapped in liposomes working in combination to confer a sustained and protective antibody response to a challenge by Leukaemia cells (WEHI-3 cells)

The Russelli vipera venom was toxoided with 2% osmium tetroxide and entrapped in liposomes (egg phosphocholine and cholesterol). The liposomes were sterilised.

The Porcine Phospholipase A_2 enzyme was entrapped in liposomes (egg phosphocholine, and cholesterol) and were sterilised.

Immunisation of mice consisted of an initial subcutaneous injection of 0.25 mls (containing 250 µg of venom) and 3 days later the mice were injected subcutaneously with 0.25 mls of porcine PLA_2 (containing 250 µg of porcine PLA_2. Boosters of each vaccine were given at 3 week intervals.

Control mice were injected with 0.25 mls of sterile physiological saline on days corresponding to test mice inoculations.

Animals

Balb/c mice (20-25 g) were used in the study. 15 mice were used in each group.

Group I - test mice

Group II - control mice

Challenge

The immunised mice and controls were challenged by intravenous injection into tail vein with approximately 5 x 10^5 leukemic cells (WEHI-3 cells) on day 30 of study.

Test mice are observed for extended life span after the death of the control mice after approximately 24 days.

Results Obtained

All control mice died of leukaemia within the allotted time span of 24 days. The venoid combination inoculation protected the vaccinated group from the cancer cell challenge and there was a 100% survival rate at day 35 when the experiment was terminated.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilise the present invention to its fullest extent. The preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the disclosure in any way whatsoever.

I Claim

1. A method of treating neoplasm in a mammal in need of such treatment, comprising administering to said mammal a therapeutic agent comprising venom and/or mammalian, plant or insect anti-serum reactive with at least one Phospholipase A_2 enzyme.

2. A method according to claim 1 wherein the anti-serum is reactive with two or more Phospholipase A_2 type enzymes.

3. A method according to claim 1 wherein the at least one Phospholipase A_2 Type enzyme is Type I, Type II, Type III or Type IV.

4. A method according to claim 1 wherein the anti-serum is either polyclonal or monoclonal.

5. A method of treating a mammal prophylactically to prevent neoplastic development, comprising administering to said mammal a therapeutic vaccine containing venom and/or mammalian, plant or insect PLA_2 enzymes or part thereof as the principal antigen component.

6. A pharmaceutical formulation containing venom and/or mammalian plant or insect anti-serum to PLA_2 enzyme or part thereof in combination with anti-serum to Phospholipase C enzyme or part thereof or inhibitory compounds to Phospholipase C for use as a therapeutic agent for the therapy of a neoplastic condition in a human or animal.

7. A method according to claim 6 wherein the inhibitory compounds to Phospholipase C is one or more of EDTA, Phenanthroline, Chloromercuribenzoic Acid, Iodoacetic Acid, and 1-oleoyl-2-acetyl-sn-glycerol(OAG).

8. A pharmaceutical formulation containing one or more venoms or venom components as antigen and/or mammalian, plant or insect PLA_2 enzyme as antigen in combination with Phospholipase C enzyme.

9. A method according to Claim 8 wherein the phospholipase C enzyme inhibitor is used in combination with the therapeutic agents of Claim I to enhance anti neoplastic and anti metastatic activity.

10. A method according to any one of Claims 1, 5, 6 and 8, wherein the administration is part of a combination therapy with other therapeutically effective agents.

11. A method according to Claims 1, 5, 6 and 8 wherein the administration is in combination with adjuvants.

12. A method according to Claims 1, 5, 6 and 8 wherein the venom is that of snakeand/or insect.

13. A method according to Claims 1, 5, 6 and 8 wherein the Phospholipase A_2 enzyme showing Phospholipase A_2 activity is obtained from more than one species of snake and/or insect, mammal or plant.

14. A method according to Claims 1, 5, 6 and 8 wherein the therapeutic agent is administered as an anti-inflammatory agent.

15. A method according to Claims 1, 5, 6 and 8 wherein the therapeutic agent is administered to prevent the occurrence of immunosuppression.

16. A method according to Claims 1, 5, 6 and 8 wherein the therapeutic agent is administered in the treating of allergic contact dermatitis, Asthma and Psoriasis and bronchitis.

17. A method according to Claims 1, 5, 6 and 8 wherein the anti-serum is administered for the treatment of physiological condition resultant from elevated levels of phospholipase A_2 products and/or metabolites.

18. A method according to claim 17 wherein the physiological condition is Schizophrenia.

19. A method according to Claims 1, 5, 6, 8 and 17 wherein the anti-serum to Phospholipase A_2 and/or C are produced synthetically by molecular imprinting of template organic molecules using these enzymes.

20. Therapeutic agents according to Claims 1, 5, 6 and 8 for treating one or more of the following:- Rheumatoid arthritis, osteoarthritis, gout, rheumatic carditis and autoimmune diseases, allergic diseases, bronchial asthma, septic shock, renal failure, pancreatis, myasthenia gravis and ocular and dermal inflammatory diseases, psoriasis, splenomegaly, cancer, metastatic spread of neoplasm, collagen vascular disease, myocardial ischemia, cellular

chemotaxis, depression, erythema, vascular permeability resultant from enhanced production of PGE_2, acne, atopic diseases, malaria, allergic conjunctivitis, schizophrenia, reiters syndrome, raynaud's phenomenon, lupus, Chron's and Graves disease.

21. A method according to Claims 1, 5, 6, 8 and 17 wherein the Fc receptor of the antibody to either Phospholipase A_2 and C used in this therapeutic method is either totally or partially removed.

22. A method according to Claims 6, 8, 19 and 21 wherein a non-toxic compound demonstrating inhibiting activity against Phospholipase C enzymes may be utilised in conjunction with the PLA_2 anti-serum to enhance its anti-neoplastic (tumour) and anti-metastatic activity.

23. A method according to Claims 1, 5, 6, 8,17 and 19 wherein the anti-serum is generated to human Phospholipase A_2 enzyme either in a mono and/or polyclonal form.

24. A method according to Claims 1, 5, 6, 8 and 17 wherein the anti-serum to Phospholipase A_2 enzyme is generated in eggs, producing antibodies which do not react with the human Compliment system.

25. A method according to Claims 1, 5, 6, 8 and 17 wherein the anti-serum to venom, mammalian, plant or insect Phospholipase A_2 is generated in mammals and extracted from the colostrum and preferably but not essentially affinity purified for use in oral administration to patients either alone or in combination with anti-serum similarly produced to human Phospholipase C enzyme components.

26. A method of inoculation of human or animal with a combination of two or more phospholipase A_2 enzymes types.

27. A method according to claim 26 where the antibody response to the inoculation confers prophylactic and/or therapeutic benefit to patient.

28. A method according to claim 27 wherein the patient is in need of a treatment for a neoplastic condition.

29. A method according to claims 26, 27 and 28 wherein the phospholipase A_2 type is Type I, Type II, Type III or Type IV.

30. A method according to claim 29 wherein the Phospholipase A_2 is obtained from venom.

31. A method according to claim 29 wherein the Phospholipase A_2 is obtained from animal or plant species.

32. A method according to claim 1, 5, 6, 8 and 26 wherein the phospholipase A_2 is synthetically produced or cloned.

33. A method of early detection of neoplastic disease by utilising the detection of enhanced PLA_2 levels in patients.

34. A method according to claim 33 wherein the detection of enhanced PLA_2 is established by the use of Lipose Diagnostic Kit.

35. A method according to claims 2, 26, 27 and 28 wherein Phospholipase A_2 type enzyme has a size of between 40-80 kDa.

36. A method of targeting cancer cells by use of Type I and/or Type II PLA_2 as targeting agent with hydrophilic tail.

37. A method according to claim 36 wherein the targeting agent is a liposome containing anti-serum to PLA_2 or conventional chemotherapy drugs.

38. A method treating parasitic and bacterial infections in mammals by the administration of a therapeutic agent containing venom and/or mammalian, plant or insect anti-serum reactive with Phospholipase A_2 enzymes

39. A method according to Claim 38 wherein the anti-serum is reactive with one or more Phospholipase A_2 type enzymes

40. A method according to Claim 39 wherein the Phospholipase A_2 Type enzymes is one of Type I, Type II, Type III or Type IV.

41. A method according to Claim 38 wherein said parasite is an haemoflagellate parasite.

42. A method as recited in Claim 41 wherein said parasite is a member of the group of haemoflagellate parasites consisting of Leishmania, Trypanosomia and Toxoplasma.

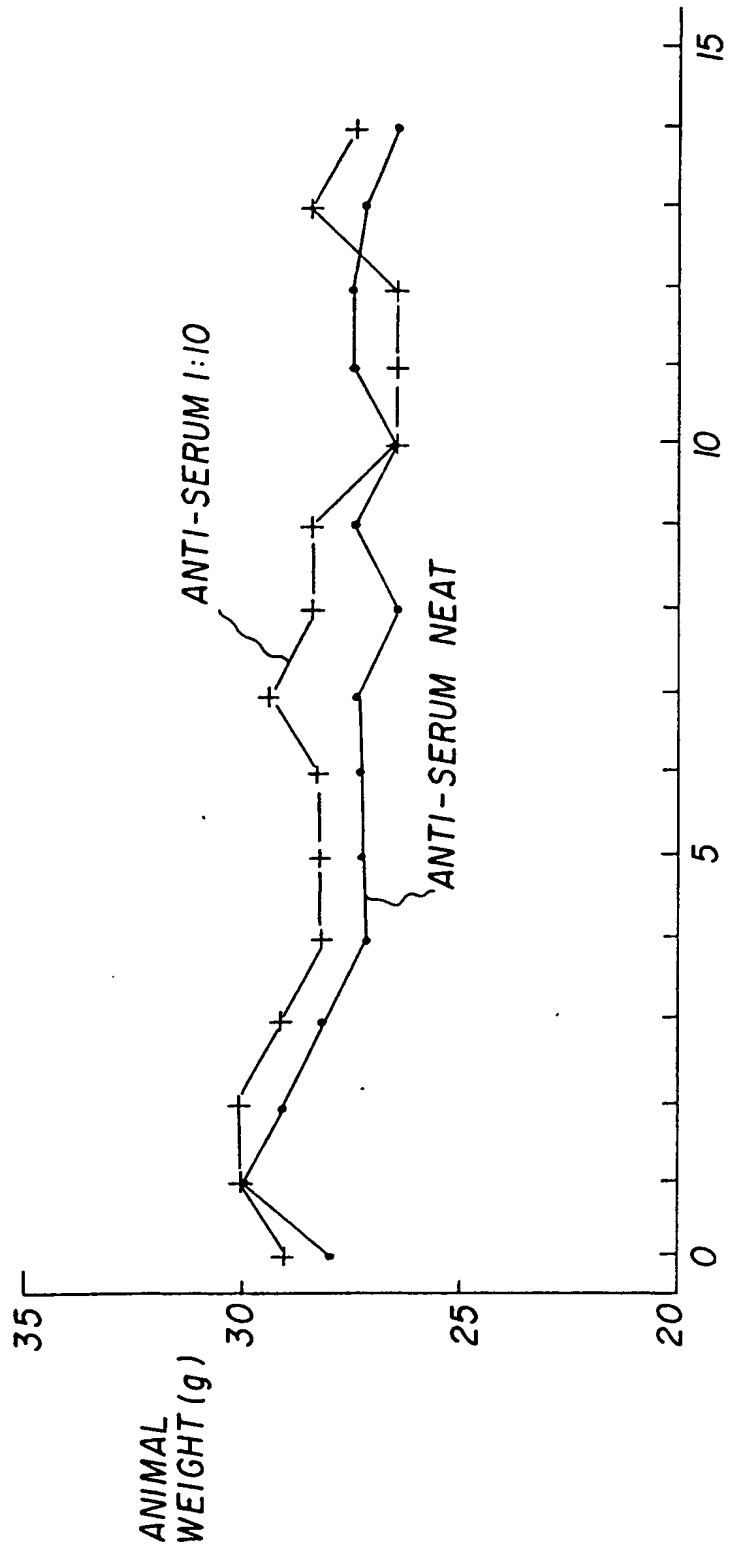

ANIMAL WEIGHT (g)
35
30
25
20
0
5
10
15
TIME (DAYS)
ANTI-SERUM 1:10
ANTI-SERUM NEAT

Fig. 1

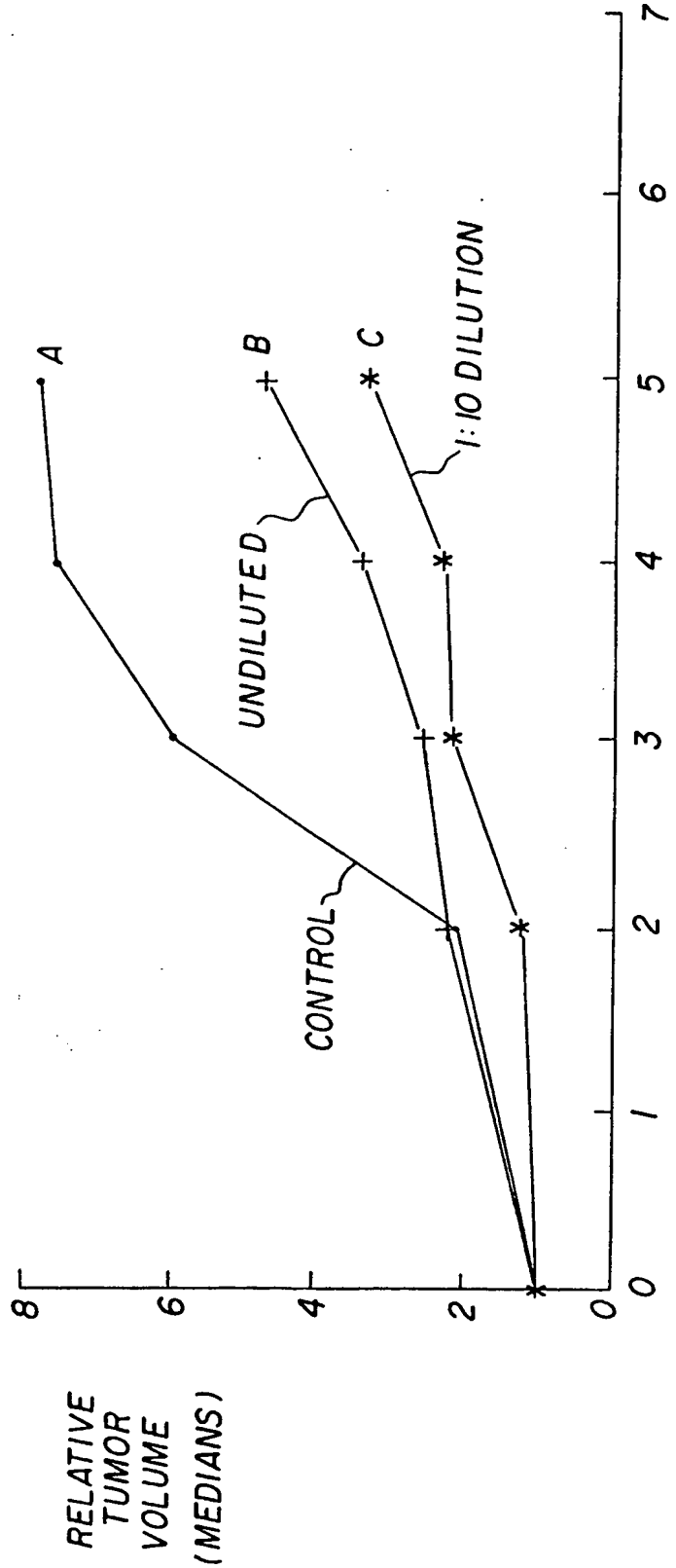
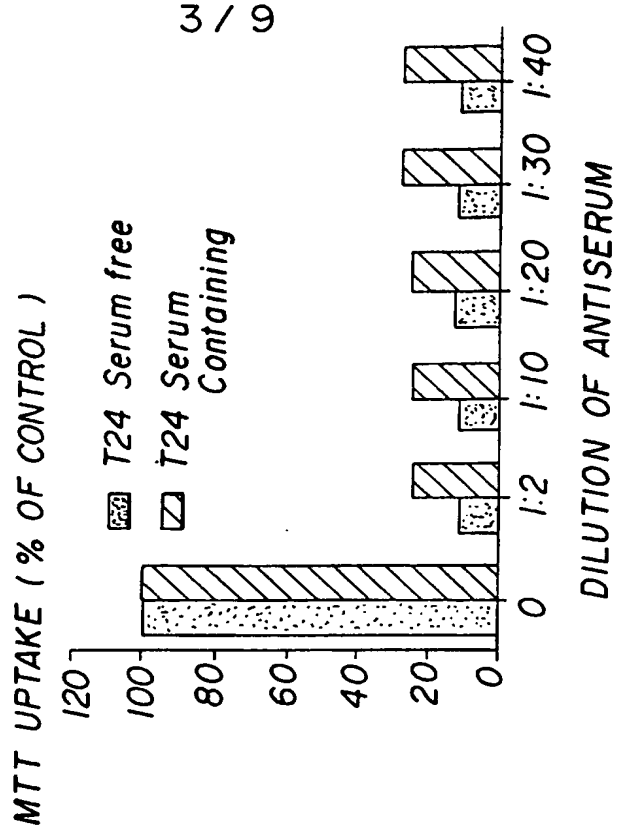


Fig. 2

*Days are measured with day zero taken as day 7 past tumour inoculation.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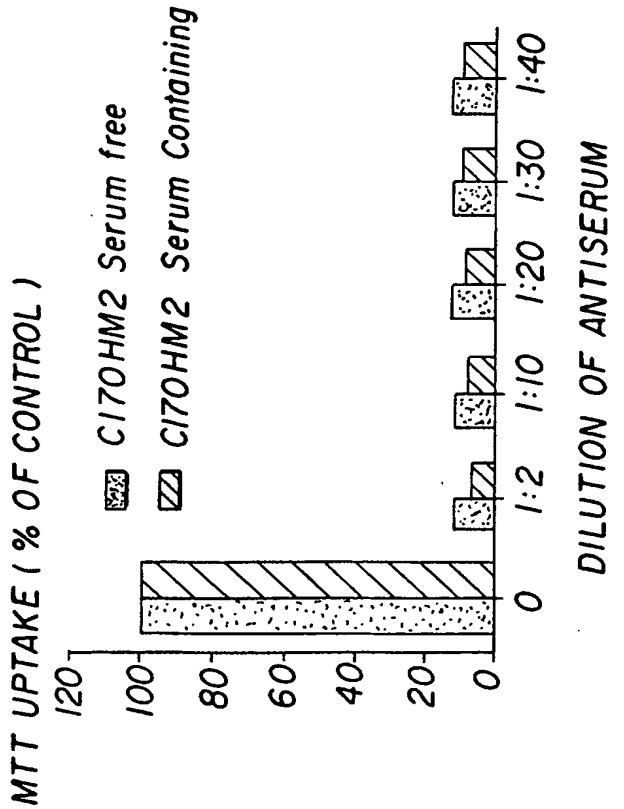
120
100
80
60
40
20
0
T24 Serum free
T24 Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3B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80
60
40
20
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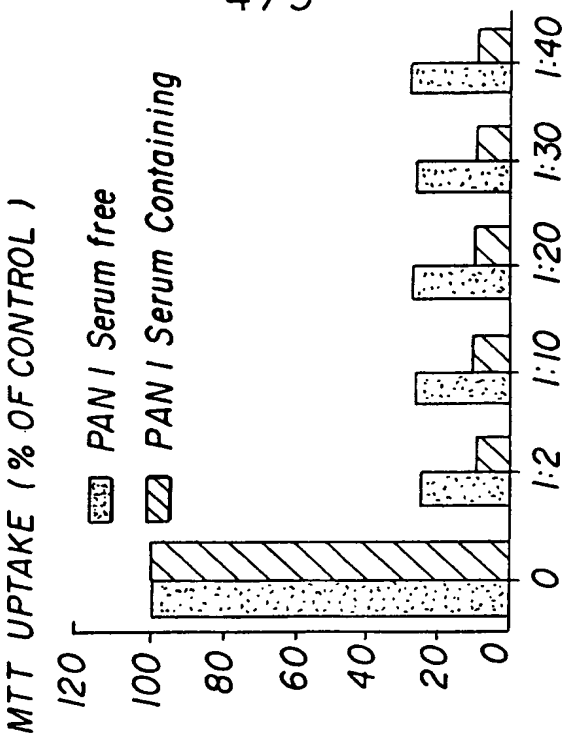
C170HM2 Serum free
C170HM2 Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3A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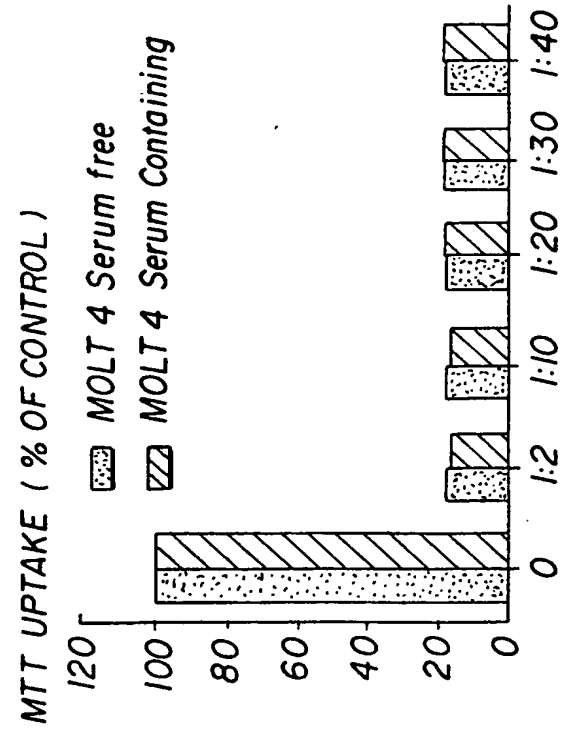
100
80
60
40
20
0
PAN I Serum free
PAN I Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3D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80
60
40
20
0
MOLT 4 Serum free
MOLT 4 Serum Containing
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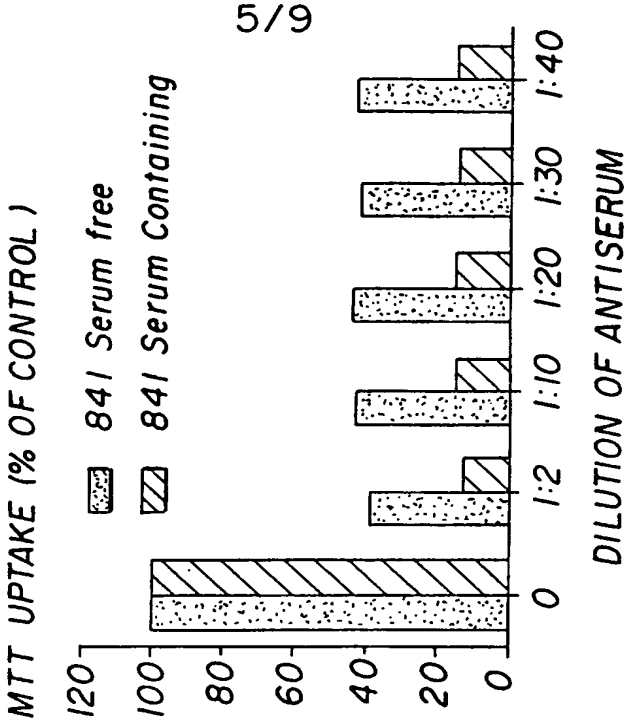
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3C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100
80
60
40
20
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841 Serum free
841 Serum Containing
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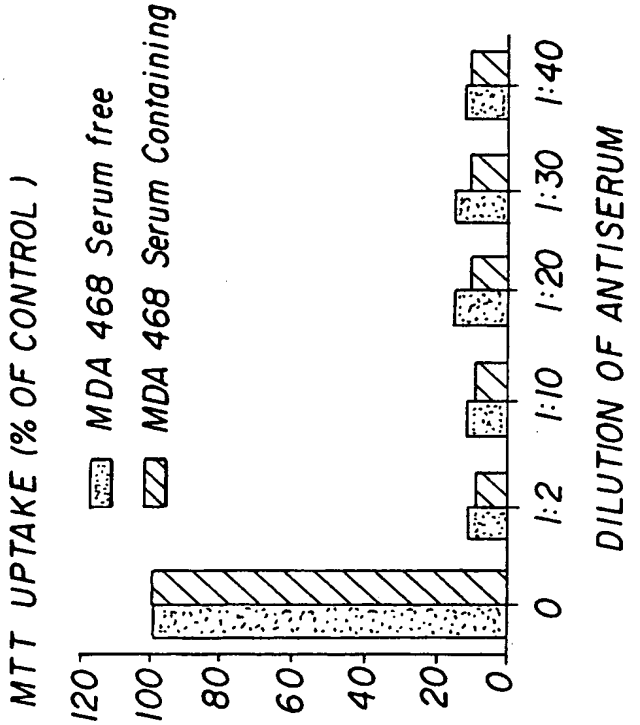
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3F


MTT UPTAKE (% OF CONTROL)
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100
80
60
40
20
0
MDA 468 Serum free
MDA 468 Serum Containing
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1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3E

Human gastric ST24

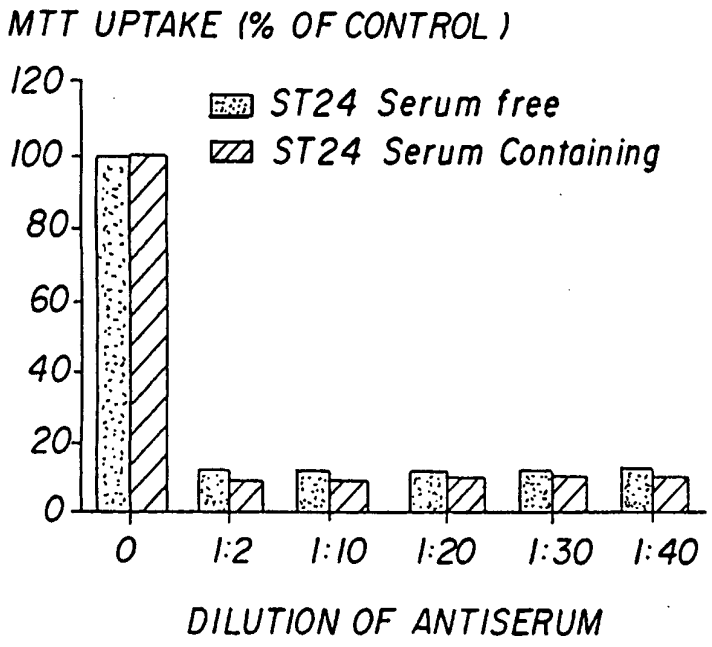


Fig. 3G

Human Ovarian OVCAR3

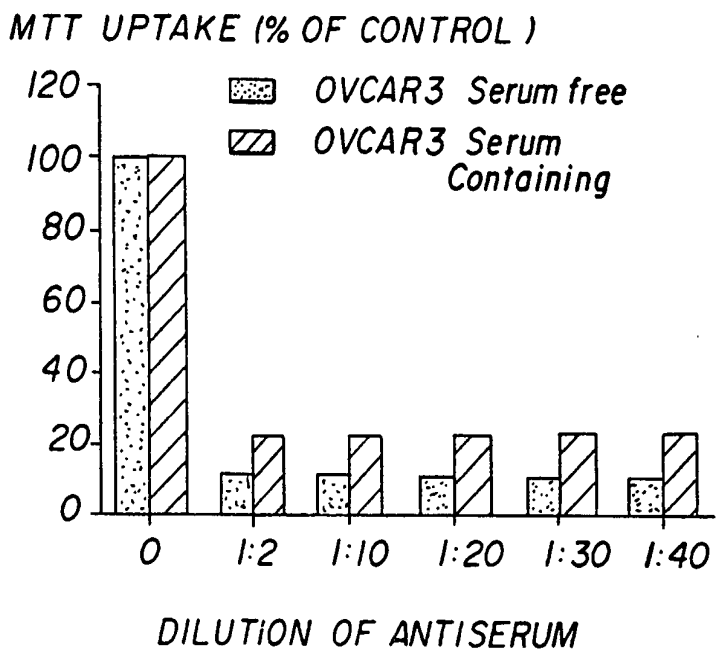
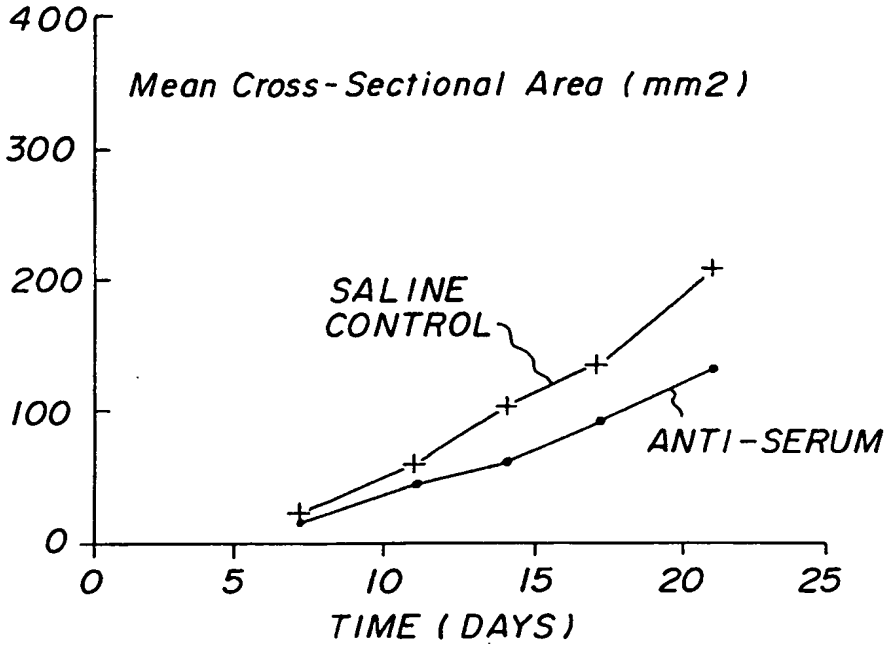


Fig. 3H


400
300
200
100
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Mean Cross-Sectional Area (mm2)
SALINE CONTROL
ANTI-SERUM
0
5
10
15
20
25
TIME (DAYS)

Fig. 4

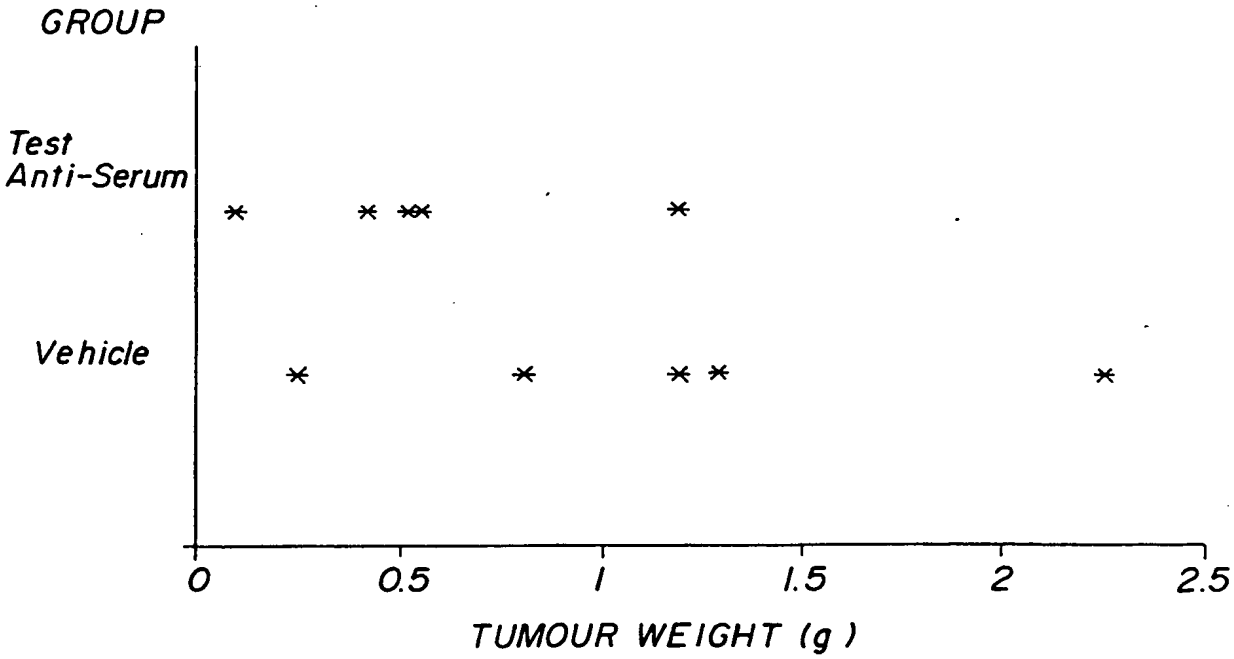

GROUP
Test Anti-Serum
Vehicle
0
0.5
1
1.5
2
2.5
TUMOUR WEIGHT (g)

Fig. 5

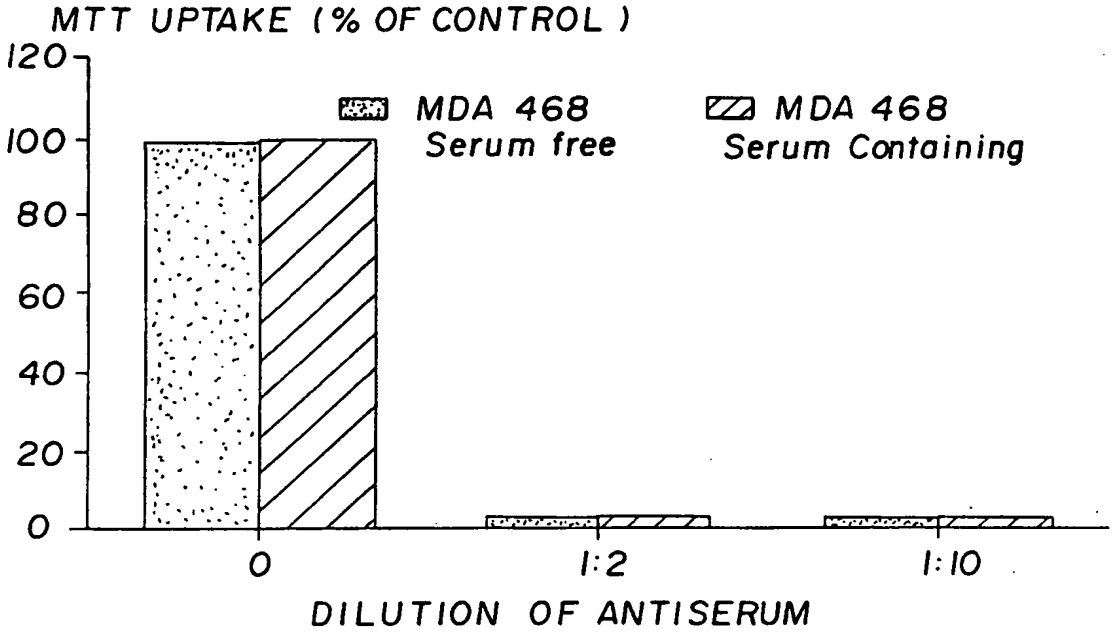
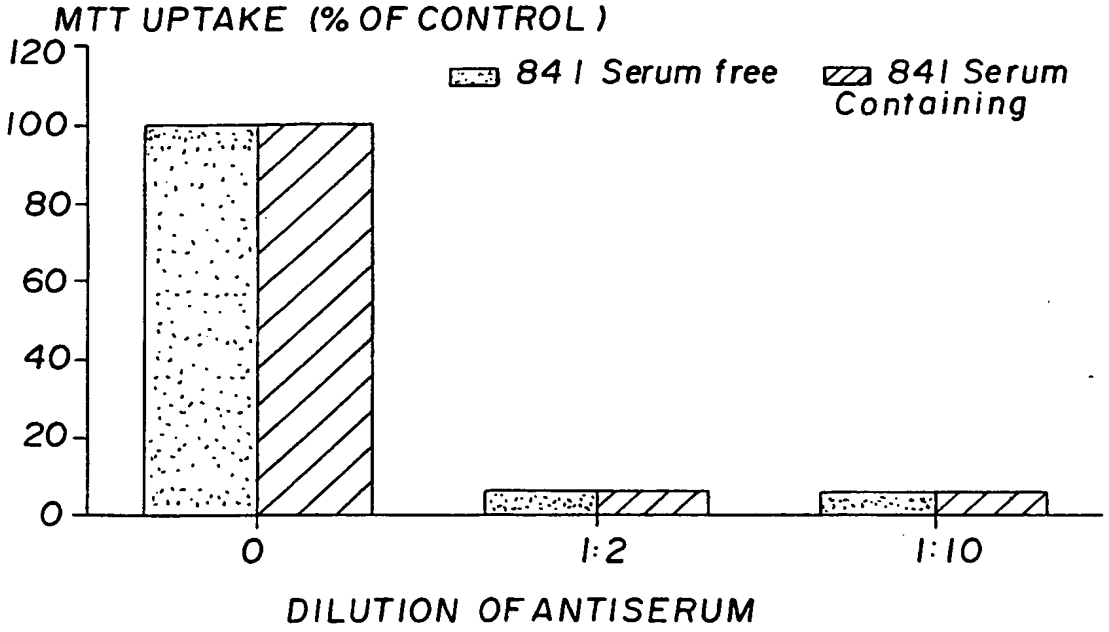

MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
MDA 468 Serum free
MDA 468 Serum Containing
0
1:2
1:10
DILUTION OF ANTISERUM

Fig. 6A


MTT UPTAKE (% OF CONTROL)
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841 Serum Containing
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1:2
1:10
DILUTION OF ANTISERUM

Fig. 6B

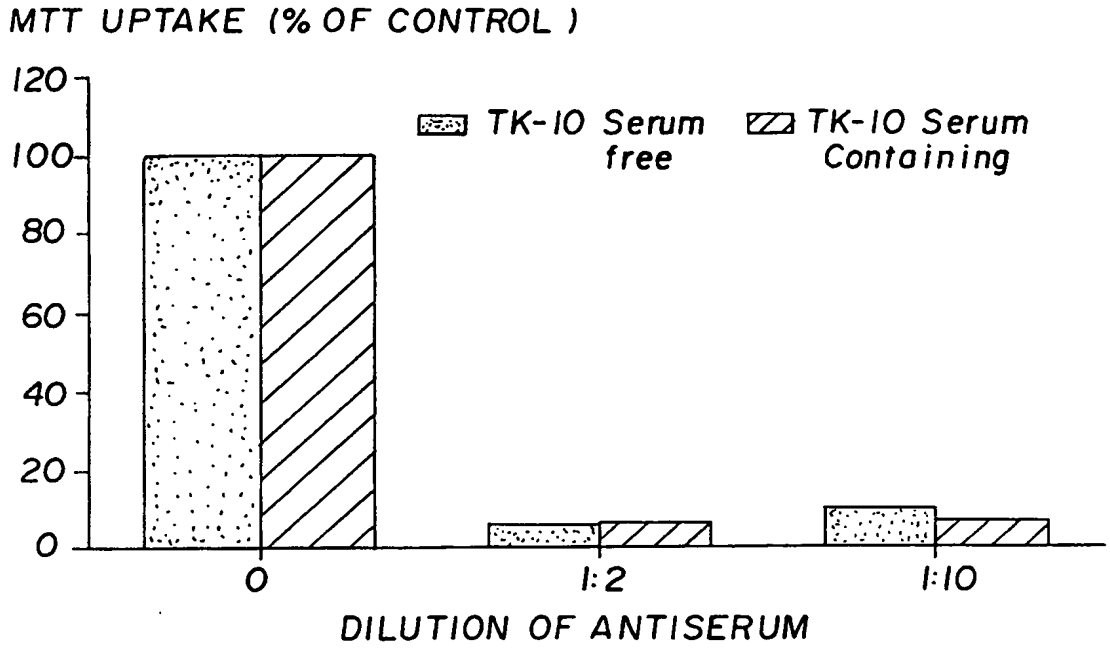
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
TK-10 Serum free
TK-10 Serum Containing
0
1:2
1:10
DILUTION OF ANTISERUM

Fig. 6C

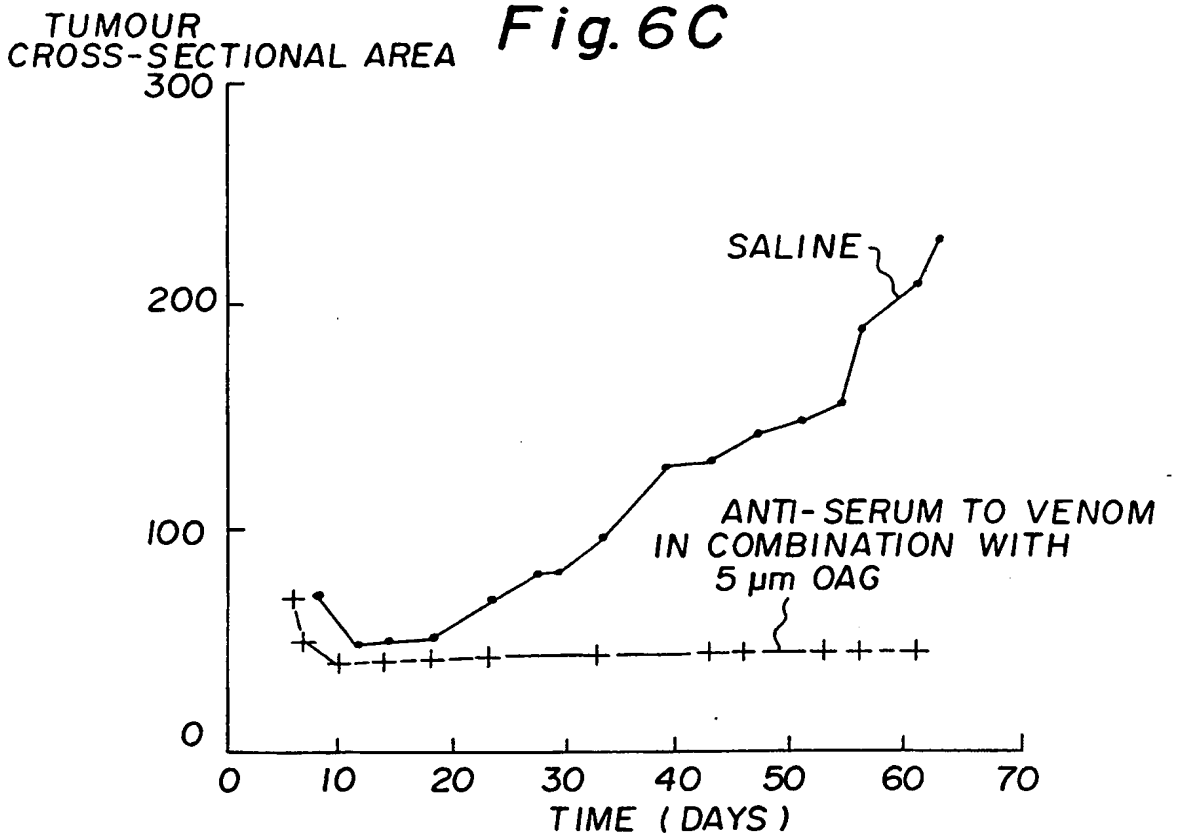
TUMOUR CROSS-SECTIONAL AREA
300
200
100
0
SALINE
ANTI-SERUM TO VENOM IN COMBINATION WITH 5 µm OAG
0
10
20
30
40
50
60
70
TIME (DAYS)

Fig. 7

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/IB 97/01091

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/58 A61K39/395 A61K38/46 G01N33/574 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 322 262 A (COMMISSARIAT À L'ÉNERGIE ATOMIQUE) 28 June 1989 see column 5, line 11 - line 15 see column 5, line 20 - line 47 see claims 12-15 ---	1-5, 10-12, 20,32
X	US 5 164 196 A (PLATA ET AL.) 17 November 1992 see the whole document ---	1,5,10, 12,20
X	EP 0 246 861 A (PLATA ET AL.) 25 November 1987 see the whole document ---	1,5,10, 12,14,20
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 1997	19. 12. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	**Authorized officer** Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 97/01091

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 459 450 A (SHIONOGI SEIYAKU KK) 4 December 1991 see the whole document	1,3,4, 14,17, 20,23,33
X	DE 41 42 552 A (BOEHRINGER MANNHEIM GMBH) 24 June 1993 see the whole document	1,3,4, 10,11, 14,17, 20,21,23
X	US 5 322 776 A (KNOPF ET AL.) 21 June 1994 see column 14, line 67 - column 15, line 43	1,16,17, 20,23, 32,35
P,X	US 5 565 431 A (LIPPS ET AL.) 15 October 1996 see the whole document	1,5,10, 12,13,20

INTERNATIONAL SEARCH REPORT

In. .ational application No.
PCT/IB 97/01091

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IB 97/01091

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: -

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 7,9-19,21-25 and 32 represent an obscurity, since, as a method claim, they, completely or partially, refer to one or more product claims.

Remark : Although claims 1-5,7,9-19,21-32,35, and 38-42 (all completely), and claims 36 and 37 (partially, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body, and although claims 33 and 34 (both partially, as far as an in vivo method is concerned) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/IB 97/01091

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 322262 A	28-06-89	FR 2623508 A	26-05-89
		DE 3877547 A	25-02-93
		JP 1206992 A	21-08-89
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